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THE UNIVERSITY OF ALBERTA

ACUTE RADIATION HYPOTENSION IN THE RABBIT: A MODEL FOR THE HUMAN RADIATION SHOCK SYNDROME

by

© MILAN THEODORE MAKALE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY IN RADIATION PHYSIOLOGY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

FALL 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ACUTE RADIATION HYPOTENSION IN THE RABBIT: A MODEL FOR THE HUMAN RADIATION SHOCK SYNDROME submitted by MILAN THEODORE MAKALE in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Zoology. Field of Study: Radiation Physiology.

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External Examiner

Date October 4, 1987

To my parents, who encouraged their son to pursue science, and whose support was extraordinary.

Abstract

This study has shown that total body irradiation (TBI) of immature (40 to 100 day old) rabbits leads to an acute fall in mean arterial pressure (MAP) 30 to 90 minutes after exposure, which takes no more than about three minutes, and often results in pressures which are less than 50% of the lowest pre-exposure MAP. This is termed acute cardiovascular collapse (ACC). ACC is often accompanied by ECG T-wave elevation, a sharp rise in ear temperature, labored breathing, pupillary constriction, bladder emptying, and loss of abdominal muscle tone. About 73% of 40 to 100 day rabbits exhibit ACC; the others and most older rabbits display gradual pressure reductions (deliberate hypotension) which may be profound, and which may be accompanied by the same changes associated with ACC. ACC and deliberate hypotension occurred in rabbits cannulated in the dorsal aorta, and in non-operated animals. The decline in MAP for all 40 to 100 day cannulated rabbits (deliberate and ACC responders) is 55.4%.

The experiments described below only involved 40 to 100 day cannulated TBI rabbits. Heart region irradiation resulted in an average MAP decline of 29.1%, with 1/15 rabbits showing ACC. Heart shielding during TBI reduced the decline in MAP to 19%, with 1/10 rabbits experiencing ACC. These results imply that the heart region, which includes the heart, part of the lungs, neural receptors, roots of the systemic vessels, and the blood, is a sensitive target.

Bilateral vagotomy reduced the decline in MAP to 24.9%, and abolished ACC. Atropine (6 mg/kg) reduced the frequency of ACC to 26%, and the decline in MAP to 41.4%. In 11/13 rabbits the voltage generated by left vagal transmission rose after TBI. The vagi appear to participate in radiation hypotension. Heart shielding together with bilateral vagotomy reduced the decline in MAP to only 9.9%, with no ACC responders.

The mean right ventricular pressure (MRVP) rose after TBI in 8/10 rabbits. In animals which displayed either ACC or steep deliberate hypotension, the MRVP rose sharply prior to the rapid decline in MAP. This suggests that the pulmonary blood flow was impeded, possibly causing right heart failure (cor pulmonale), and consequent cardiovascular collapse.

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I very much wish to thank my supervisor, Professor R. F. Ruth for instilling in me various professional standards, for instructing me in how one proceeds systematically, and for his compassion and limitless patience. I also wish to thank Mr. R. J. Gardner of the Radiation Research Centre at the University of Alberta, for his patient instruction and for his very generous help, without which much of this work could not have been done. I am also very grateful to Ms. L. Morningstar for her constant support, and for typing the tables and assisting with the photocopying. I would also like to express my deep appreciation to the following people who contributed substantially to this project, and/or my personal professional development: Professor G. R. Freeman, Mr. L. Coulson, Mrs. E. Dimitrov, the late Mr. John Lund, Mr. J. Bouvier, Dr. J. D. Chapman, Professor R. Stein, Dr. N. Gee, Mr. T. Germaine, Mr. J. Hendrikson, Dr. J. W. Scrimger, Dr. S. Usiskin, Mrs. K. Evenson, Mr. M. Henderson, Dr. J. Russell, Mr. H. Lamont, and Mrs. C. Miner. I am extremely grateful to the Alberta Heritage Foundation for Medical Research (AHFMR) for providing me with a stipend and a research allowance.

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List of Abbreviations

ACC = acute cardiovascular collapse

ACh = acetylcholine

AChE= acetylcholinesterase

AEC = Atomic Energy of Canada Limited

AET = S-beta, 2-aminoethylisothiuronium bromide hydrobromide

AMB = atropine methyl bromide

AMN = atropine methyl nitrate

AS = atropine sulphate

CCI = (W. W.) Cross Cancer Institute (Edmonton, Canada)

cGy = centiGray = 1 rad

Co^{60}= cobalt 60

DAM = diacetylmonoxime

EACA= epsilon amino-N-caproic acid

ECG = EKG = electrocardiogram

Gy = Gray = 100 rad

Hz = Hertz = cycles per second, eg., 1 kHz is 1,000 cycles/second

IVC = inferior vena cava

kev = kv = thousand electron volts

LHBI= lower half body irradiation

MAP = mean arterial pressure

MeV = million electron volts

MRVP= mean right ventricular pressure

PAM = pyridine-2-aldoxime methiodide = pralidoxime chloride

PAP = pulmonary artery pressure

RRC = Radiation Research Centre (University of Alberta)

RV = right ventricle, or right ventricular

SBTI= soyabean trypsin inhibitor

TBI = total body irradiation = whole body irradiation

UHBI= upper half body irradiation

I. Introduction

Just four years after their discovery by Wilhelm Roentgen in 1895, X-rays were used by a Swedish doctor, Tor Steenbeck, to cure a case of skin cancer (Ellinger, 1957). Subsequently, the clinical use of radiation expanded rapidly and showed significant promise. But as early as 1897, a paper by D. Walsh, a physician, appeared in the British Medical Journal, describing adverse constitutional reactions developing in irradiated individuals (Walsh, 1897). In one notable case referred to by Walsh, the patient sustained a substantial exposure of the head over the course of a week. The author did not indicate whether any other parts of the body were exposed, but given the fact that the X-ray tubes of that period produced very wide radiation fields, it is likely that the patient received a significant dose to the entire upper half body. [1] This individual suffered fever, vomiting, giddiness, headache, diarrhea, and prostration. Within the next few decades, similar accounts by other investigators extended Walsh's description, and it became apparent that even during, or within hours of a single radiation exposure, any or all of, fever, nausea, vomiting, dizziness, mental confusion, prostration and diarrhea could occur (Lange, 1915; Pfahler 1916; Hall and Whipple, 1919). [2]

---

[1] This conclusion was arrived at by Professor L. Stephens-Newsham, upon reading Walsh's paper. Dr Stephens-Newsham is Professor Emeritus of the Faculty of Pharmacy, and past chairman of the SLOWPOKE reactor committee at the University of Alberta.

[2] It is not clear from the cited literature whether the exposures leading to these effects were total body, upper half body, lower body, or localized.

In 1958 Gerstner categorized these manifestations as belonging to the condition termed the "prodromal phase of acute radiation sickness" (Gerstner, 1958).

In the decades following World War II, several nuclear plant mishaps occurred in which workers were heavily irradiated. Two of these accidents revealed that shortly after radiation exposure, humans may suffer profound, lethal hypotension in addition to the effects described by Walsh and his contemporaries (Shipman et al, 1961; Karas and Stanbury, 1965). In these particular cases, two individuals died, both within about forty-eight hours of exposure, and in each instance death was attributed to cardiovascular collapse. Both accidents and the case histories, which are well documented, are summarized below.

In 1958, at Los Alamos, New Mexico, and in 1964, at a United Nuclear Corporation plant in Rhode Island, solutions containing fissionable material reached criticality and flash exploded, liberating gamma rays and fast neutrons. The Los Alamos victim received an average whole body dose of 4,500 centi-Grey (cGy), with about 12,000 cGy to the upper abdomen, while the Rhode Island subject sustained an average whole body dose of about 8,800 cGy. The men died at 35 and 49 hours, respectively, following exposure. Neither individual suffered injury caused by the heat or the force of the blast. Upon admission to the hospital, the Los Alamos patient had a blood pressure of 40/10, and although this was raised, normotensive levels were never attained. The Rhode

Island patient was initially hypertensive, but within six hours of irradiation his pressure began to steadily drop. Despite constant venous infusion of fluids and vasopressors in both patients, the blood pressure tended to edge down, and with both men, the diastolic pressure was unobtainable for several hours prior to death. Autopsy disclosed edema of the skeletal muscle, gastrointestinal tract, lungs, right heart, and liver. The brain of the Los Alamos victim was swollen and edematous, but exhibited relatively mild microscopic changes; the attending pathologist postulated that the swelling and edema were explainable on the basis of right heart failure, and consequent cerebral passive congestion and anoxia. The brain of the Rhode Island victim appeared grossly normal, and showed minimal microscopic changes. No mention was made in either autopsy report of the condition of peripheral neural structures. Despite the fact that the radiation doses were very high and might be supposed to cause death from neurologic damage, the authors of each case report suggested that death was due to cardiovascular effects, and that any neurologic manifestations stemmed from cardiovascular changes. In 1983 a reactor accident at the Constituyentes Atomic Center in Argentina exposed one man to an average whole body dose of 1,400 cGy of fast neutrons and approximately 500 cGy of gamma rays; well below dose levels thought to cause neurologic death. The victim died at about 49 hours after the accident (Jordan, 1983). The patient exhibited symptoms of radiation sickness, with some nervous disorders, although hypotension was not mentioned in the preliminary report. The cause of death was indicated as "severe inflammation of the lungs". Although at the time of this writing it has not been possible to learn if this man suffered hypotension, the report is worth noting because the time of survival matches those of the Los Alamos and Rhode Island victims. [3]

During the past four decades upper half body irradiation (UHBI) has been used intensively to treat various malignancies (Salazar et al, 1978; Salazar et al, 1980; Van Dyk et al, 1981; Urtasun et al, 1983). Commonly, patients suffer nausea, vomiting, fever, and often exhibit a detectable diminution in arterial blood pressure (Salazar et al, 1978; Urtasun et al, 1983). Figure 1 portrays the decline in systolic and diastolic blood pressure with time after exposure, for 29 lung cancer patients subjected to UHBI with 800 cGy of X-rays. The pressures were reduced by about 1.5 hours following irradiation, and by about 8 hours, had declined to a minimum which was around 80% of the respective pre-irradiation levels. For 9 lower half body irradiated patients (LHBI), the pressure did not change significantly. When the incidence of hypotension with UHBI was compared to that for LHBI using Fisher's Exact Probability test, the probability of hypotension developing with UHBI and not with

---

[3] No published report on the medical aspects of this case is yet availible, and according to Dr C. C. Lushbaugh (personal communication) of the Oak Ridge National Laboratory at Oak Ridge, Tennessee, it seems that no autopsy was performed. It is unclear whether the patient was hypotensive throughout the post-exposure peroid, or just pre-terminally.

LHBI was 0.006.[4] The hypotension caused by radiotherapy can be substantial, and death indirectly resulting from this syndrome, has been reported for 2 patients which were subjected to UHBI with 800 cGy of 10 MeV X-rays (Salazar et al, 1978). Both of the individuals had a history of heart disease. The severe hypotension elicited by radiotherapy caused myocardial infarct formation, which later led to a second, fatal infarct.

Between the turn of the century and the end of World War II, it appears that few reports were published describing studies of radiation induced cardiovascular changes in animals. Nonetheless, in 1924, thorough work by Swann demonstrated that some New Zealand White rabbits irradiated over the chest and abdomen with at least 4,750 cGy of X-rays at about 950 cGy/minute, exhibited a deep decline in arterial pressure which developed gradually over the first post-irradiation hour (Swann, 1924).[5] The development and use of nuclear weapons spurred research on the early effects of radiation exposure. Results from Painter and her colleagues in 1946, and from Brooks and his co-workers in 1956, both using more modern equipment and methods than pre-war investigators, revealed that New Zealand white rabbits subjected to total body irradiation (TBI) with between 600 and 800 cGy of 200 or 260 kvp X-rays at 10.6 or 12 cGy/minute, exhibited an approximately 50% decline in MAP by about two hours post-exposure (Painter et al, 1946; Brooks et al, 1956). Several of the animals died within this time. Through the work of several investigators, rhesus monkeys (*Macacca mulatta*) have been irradiated over the trunk or the whole body, with between 1,000 and and 6,600 cGy of Gamma rays or 6 MeV X-rays delivered at 110 to 425 cGy/minute, or with 2,500 to 5,000 cGy of mixed gamma-neutron radiation delivered in a reactor pulse lasting a fraction of a second. The animals experience during or just after exposure, a precipitous drop of at least 50% (of the pre-exposure value) in mean aortic blood pressure (Chapman, 1968; Young, 1968; Miletech and Strike, 1970; Turns et al, 1971; Turbyfill et al, 1972; Doyle et al, 1974; Bruner, 1977; Alter et al, 1983). The pressure recovers in a few minutes, then declines gradually, with death often supervening within 24 hours of the higher doses of radiation. Within minutes of irradiation, the monkeys are less able to perform previously learned mental tasks (Chapman, 1968; Young, 1968; Miletech and Strike, 1970; Bruner et al, 1975; Turns et al, 1971; Turbyfill et al, 1972; Doyle et al, 1974; Bruner, 1977). The minimum dose which can produce a drop in mental performance in the rhesus monkey is 500 cGy of Co^{60} at 163 to 233 cGy/minute (Bruner et al, 1975). Rats which are total body irradiated (TBI) with 700 cGy of neutrons or with 600 to

---

[4] The data for this analysis was generously supplied by Dr R. C. Urtasun of the W. W. Cross Cancer Institute in Edmonton, Alberta.

[5] In his paper Swann did not give radiation doses, but did describe the apparatus and conditions used in his irradiations. The doses indicated here were estimated by Dr L. Stephens-Newsham, Professor Emeritus, of the Faculty of Pharmacy, and member of the SLOWPOKE reactor committee, at the University of Alberta.

2,500 cGy of 250 or 200 kvp X-rays at 15.4 to 123 cGy/minute, experience an appreciable reduction in MAP within ~~6 hours~~ of exposure (Weber and Steggerda, 1949; Montgomery and Warren, 1951; Phillips and Kimeldorf, 1963; Wykoff, 1972). TBI of 3-4 day and 6-8 month old chickens with 1000 or 1200 cGy of 200 or 250 kv X-rays at 35 to 43 cGy/minute, leads within hours, to a 75% reduction in MAP in young birds, and a 20-25% reduction in MAP of adult birds (Stearner et al, 1955; Stearner et al, 1956). Within 9 hours of exposure many of the birds die (Jacquez and Karnofsky, 1950; Stearner et al, 1955; Stearner et al, 1956).

Three different mechanisms have been suggested to explain radiation induced hypotension. These proposals, which are not necessarily mutually exclusive, have generally guided the study of this syndrome, and are reviewed below. First, a commonly and long-held notion is that TBI or UHBI irradiation with several hundred cGy, causes widespread tissue damage, thus triggering the release of large amounts of vasoactive substances into the interstitial fluid and blood. This liberated material, thought to mainly include histamine, would elicit extensive vasodilatation of peripheral vascular beds, resulting in a deep blood pressure drop. Markedly elevated levels of plasma histamine have been reported for irradiated rhesus monkeys, rabbits, and rats (Painter et al, 1946; Weber and Steggerda, 1949; Doyle et al, 1974; Doyle and Strike, 1975; Alter et al, 1983). Rise in plasma histamine has been reported for patients undergoing radiotherapy to the cervical region (Lasser and Stenstrom, 1954). Painter et al found that injection with histaminase failed to prevent early shock and death of irradiated rabbits, but treatment with the H1 receptor antihistamine Benadryl (diphenhydramine hydrochloride) reduced the extent of the decline to 29 mm Hg, as compared with 50 mm lowerings typical for untreated animals (Painter et al, 1946). However the compound was not completely effective, and the authors cautioned that Benadryl may have autonomic effects. Benadryl is known to have powerful sedative effects (Goodman Gilman et al, 1980). Furthermore, Painter and her co-workers found that intravenous injection with 0.5 mg/Kg of atropine prior to exposure provided a level of protection from the pressure decline which was comparable to that with Benadryl. But, when Benadryl and atropine were injected together, the decline in blood pressure was about equal to that recorded for rabbits which had been injected with either drug alone. The absence of a synergistic effect was taken to imply that atropine and Benadryl acted on the same, rather than on different processes. Furthermore, all four animals injected with atropine before 800 cGy TBI, the 30 day LD 50 for rabbits, survived longer than 30 days. It should be noted however, that Painter tested ostensibly effective doses of Benadryl in only two rabbits, atropine in four animals, and atropine together with Benadryl in six individuals--- these experiments can therefore not be regarded as decisive. However, Larkin (1949) reported that

0.1 ml of a 0.00045 Molar solution of atropine extended the mean survival time of mice irradiated with 1152 cGy, from 5 days to 12 days.

Pre-irradiation treatment of the rhesus monkey with H1 receptor antihistamines either alone, or together with an H2 antihistamine, has blocked the initial, sudden pressure drop (Doyle et al, 1974; Alter et al, 1983). The gradual pressure decline which begins within a few minutes after recovery from the sudden drop was diminished, but not prevented, by antihistamines. Furthermore, several monkeys died between 100 minutes and 35 hours of irradiation despite pre-treatment with antihistamine(s) (Doyle et al, 1974). Weber and Steggerda (1949) found that histamine was elevated in the blood of rats after TBI, but that 4-5 hours after exposure, histamine in the blood was no longer measurable, even though the blood pressure was still low, and declining further. The mechanism by which the early phase of hypotension in the rhesus was blocked is unknown; it should be noted that H1 antihistamines have central nervous system effects. It is of interest that the decline in mental performance was significantly lessened with H1 antihistamine (chlorpheniramine) treatment, but when the blood pressure was maintained at near normal levels with norepinephrine, the mental task competence was not at all improved (Turns et al, 1971). This suggests that the antihistamine was operating, at least in part, on neural mechanisms, and not just by maintaining adequate blood pressure. Although norepinephrine has markedly reduced the depth of irradiation hypotension in the rhesus, the doses needed to achieve this were high, and in one report the average post-irradiation survival time for the treated animals was significantly diminished (Turns et al, 1971). This is consistent with the finding that epinephrine was of little benefit to the nuclear plant victims (Shipman et al, 1961; Karas and Stanbury, 1965). The use of norepinephrine and epinephrine in irradiation hypotension is dealt with in the Discussion. It is possible that the post-irradiation rise in circulating histamine is due to the release of this substance from mast cells. Accordingly, in one experiment, monkeys which underwent several rounds of treatment with the mast cell histamine depleter, compound 48/80, showed no significant rise in the plasma histamine concentration following whole body irradiation (Doyle and Strike 1975). However, no reference is made in any of the monkey papers to the blood pressure of animals which were treated with 48/80 prior to irradiation--- it would be highly useful to learn whether monkeys which are depleted of mast cell histamine exhibit the radiation induced drop in blood pressure. To test whether a blood borne factor is involved in the hypotension, Painter et al (1946) replaced about 50% of the blood of non-irradiated rabbits with blood taken from irradiated, hypotensive animals. This caused an approximately 25% pressure reduction in the recipients--- but in one case injection with blood from a non-irradiated rabbit into a non-irradiated recipient elicited a 12%

pressure reduction. Brooks et al (1956) injected small quantities of reconstituted plasma taken from irradiated rabbits into non-irradiated animals. The maximal pressure fall averaged 6 mm Hg, which the authors indicate barely surpassed the blood pressure measurement error. Subsequently, Gerstner conducted a careful cross circulation experiment between non-exposed rabbits and whole body irradiated partners (Gerstner 1957). None of the non-irradiated animals exhibited any decline in blood pressure. The results for histamine tests are not conclusive with respect to irradiation hypotension; the information gathered thus far does indicate that histamine may play an important role, but the nature of this putative participation is not clear, and antihistamines alone do not constitute satisfactory therapy.

The second general mechanism proposed to account for radiation induced hypotension is that radiation either directly damages the peripheral vasculature, or causes the release of lytic enzymes which attack the peripheral blood vessels. This causes the vessels to become more permeable, and thus facilitates the extravasation of whole blood or plasma, resulting in hypovolemic shock. Studies relating to this idea have involved labelling plasma constituents in the rabbit with vital dyes or radioisotopes, and irradiating local patches of skin. Several workers have found that between about 30 and 90 minutes following exposure with between 450 and 4,000 cGy of X-rays, the irradiated skin exhibits a clearly increased concentration of plasma marker, heralding local dilatation and/or increased vascular permeability (Rigdon and Curl, 1943; Painter et al, 1946; Mount and Bruce, 1964; Jolles and Harrison, 1966; Eassa and Casarett, 1973). Furthermore, treatment with enzyme inhibitors such as epsilon-N-aminocaproic acid (EACA), and soyabean trypsin inhibitor (SBTI), has significantly reduced the accumulation of plasma markers in locally irradiated skin (Jolles and Harrison, 1966; Eassa and Casarett, 1973). Stearner and Azuma claimed that injection of chicks with 75 mg/Kg SBTI prior to whole body exposure reduced the extent of hypotension, and nearly abolished early mortality (Stearner and Azuma, 1968). However, Stearner's experiments do not appear to have been duplicated elsewhere, and no further work seems to have arisen during the 19 years following her papers. Also, perfusion experiments with isolated rabbit ears have revealed that the rate of perfusion drops after irradiation with between 20 and 40 Gy, and that the post-exposure weight of the ears is not increased (Gerstner and Brooks, 1955). This suggests that vasodilatation occurred in these ears, but that vascular permeability was not changed (Gerstner and Brooks, 1955; Mount and Bruce, 1964). Stearner et al found that following TBI of both 3-4 day and 6-8 month old chickens, the plasma volume fell and the hematocrit was elevated (Stearner et al, 1958). But, in general, blood volume and hematocrit measurements for whole body irradiated rabbits and rats have not provided any evidence for the extravasation of whole blood or plasma

(Painter et al, 1946; Montgomery and Warren, 1951; Phillips and Kimeldorf, 1963). However, Caster et al (1958) found that the plasma volume of various structures was increased in rats after 700 cGy TBI. Nevertheless, the blood volume, hematocrit, and ear perfusion results, as far as mammals are concerned, oppose the hypothesis that radiation causes the peripheral vasculature to become more permeable, but do not contradict the possibility that significant vasodilatation occurs. In theory, peripheral vasodilatation could be produced by any of the following processes, operating singly or in any combination: (1) direct radiation effects on the vascular smooth muscle and/or local smooth muscle innervation, (2) the release of vasoactive factors from radiation damaged tissue, or (3) efferent neural activity stimulated by irradiation of the nervous system. It appears that no study has been published in the open literature in which skin concentrations of plasma markers were measured during irradiation hypotension.

The third mechanism postulated for radiation hypotension rests on the possibility that whole or upper half body exposure affects the nervous system to a degree which is sufficient to seriously alter the behavior of the autonomic system. Radiation may destabilise neural components, and since autonomic nerves richly supply the cardiovascular system, certain patterns of nervous outflow could degrade cardiac function, and/or generate extensive peripheral vasodilatation, thereby causing a fall in blood pressure.

Although the nervous system has been regarded by some workers to be quite resistant, many laboratories have histologically demonstrated changes in the brain of animals within hours or days of exposure to between 3,000 and 14,000 cGy (Lyman et al, 1933; Hempelmann et al, 1952; Clemente and Holst, 1954; Alvord and Brace, 1957; Gerstner et al, 1957; Gerstner and Kent, 1957; Wilson, 1960; Kagan and Brownson, 1962). Changes observed for the brains of irradiated animals include pyknosis of cells, vasculitis, meningitis, and increased size of cerebellar Purkinje cells. Several studies have demonstrated edema of the brain occurring within hours of head or whole body irradiation with between 4,200 cGy and 14,000 cGy (Gerstner et al, 1956; Gerstner and Kent, 1957; Leith, 1972). In contrast, the peripheral nervous system appears to be highly resistant to radiation (Griffith, 1934; Sato, 1978) However, Suzuki (1931) found that irradiation of mature female rabbits to expose the inferior mesenteric ganglion has resulted in atrophy of that structure (Suzuki, 1931). Also, Swann (1924) reported that in anesthetized rabbits irradiated over the chest and abdomen, the sympathetic and parasympathetic nerves were rendered more sensitive to electrical stimulation following relatively low doses of radiation, and less sensitive following higher doses. In any event, cats, rats, and rhesus monkeys are less responsive to norepinephrine after irradiation (Ryzewski, 1962; Miletech and Strike, 1970; Wykoff, 1972). It was found that the Rhode Island and Los Alamos accident victims responded poorly to norepinephrine (Shipman et al, 1961; Karas and Stanbury, 1965). Stearner injected 3-4 day chicks intramuscularly with epinephrine, 5 or 10 mg/kg in peanut oil, either 5 minutes before or 5 minutes after 600 to 2000 cGy of X-rays (Stearner et al, 1954). The epinephrine delayed, but did not prevent the hypotension.

A range of experiments has provided evidence for immediate functional changes in the nervous system caused by low dose irradiation; representative studies are described below. It has been reported that TBI with 2,000 cGy at 10 to 100 cGy/minute causes presynaptic inhibition in the spinal cord of decerebrate or anesthetized cats to increase in strength and in duration (Barnes, 1967). Automated electroencephalogram (EEG) analysis has disclosed electrocorticographic changes in rats receiving 700 cGy whole body (Caster, 1958). Definite EEG changes have been recorded immediately following whole body irradiation of the rhesus monkey with 1000 cGy of Co^{60} gamma rays (Brooks, 1956). Cats, with chronically implanted electrodes, exhibit spike discharges from the hippocampus within about one hour after 400 cGy of 250 kvp X-rays to the head, and also after 400 cGy or 200 cGy TBI (Gangloff, 1959; Gangloff et al, 1960). Rabbits and cats which were head irradiated with 400 cGy of X-rays at 60 or 90 cGy/minute exhibited changes in hippocampal activity; five animals showed increased spike discharges, and five showed decreased spike discharges (Gangloff, 1962). Bassant and Court (1978) found that rabbits subjected to TBI with 450 cGy of X-rays at 14 cGy/minute, exhibit highly disturbed hippocampal cellular activity. Also, Gueneau et al (1979) have reported that rabbits which were subjected to either TBI, or brain irradiation, with doses of at least 1.5 Gy, exhibited pyknosis of the differentiated and undifferentiated hippocampal granule cells at 3 hours after exposure. The mature hippocampal granule cells exhibit light spots within the nuclei at 1 hour after exposure. These changes were most extensive with doses above 4.5 Gy. They occurred in both immature and adult rabbits, but were more pronounced with younger animals. Rabbits irradiated with 12,500 cGy to the head immediately experience drowsiness, and they die within six days of exposure (Gerstner et al, 1955). A Soviet report claimed that following TBI of the anesthetized rabbit with 1,000 cGy of X-rays, the amplitude of vagal discharges increases substantially, and peaks at about one hour after exposure (Livanov and Biryukov, 1958). Painter et al (1946) reported that rabbits which had both cervical vagi transected prior to whole body irradiation with 600 cGy of 200 kvp X-rays, experienced hypotension which was roughly half as deep as that for rabbits with intact vagi. It was also observed that pre-irradiation intravenous injection with 0.5 mg/Kg of atropine lessened the extent of hypotension by about 50%. This and the double vagotomy result, while arising from preliminary efforts, point to an autonomic role in radiation hypotension. Brooks et al (1956) noted that whole body irradiated, hypotensive rabbits exhibited tachypnea, tachycardia, and cardiac arrythmia--- manifestations which the authors stated were suggestive of vagal stimulation. Chemoreceptor trigger zone ablation has been shown to abolish post-irradiation emesis in dogs which were whole body exposed to 800 cGy of 260 kvp X-rays at 24 cGy/minute (Wang et al, 1958). Montgomery and Warren (1951) spinalectomized rats at the C6 level and then subjected them to TBI with 1,500 to 2,500 cGy of 200 kv X-rays. Spinalectomy caused a significant reduction in MAP which was not deepened by irradiation. The spinalectomized, irradiated rats did not experience greater hypotension than did non-irradiated, spinalectomized animals, and their arterial pressures were higher than those of non-spinalectomized, irradiated rats. The authors stated that these results preclude a key role for histamine in radiation hypotension, and do implicate a major contribution by the nervous system. It should be cautioned however, that spinalectomy radically affected the rats, and any animal so treated can hardly be expected to react in a normal manner. The results are suggestive of a neural role in radiation hypotension, and do make it seem less likely that histamine would produce this syndrome through peripheral vasodilatation, but key participation by histamine is not ruled out by this work.

To summarize the findings of the radiation work which relates to the nervous system, it seems that various neural effects can follow even moderate whole, or upper half body exposures, and that radiation induced changes in the nervous system may be connected with the early drop in blood pressure. However, some key results in this area contradict each other. For example, autopsy of the nuclear plant accident victims disclosed no significant microscopic changes in the brain, while animal tests have revealed evidence of microscopic damage within hours of exposure to several hundred cGy (Shipman et al, 1961; Karas and Stanbury, 1965). Still, in the accident victims the brain and meninges were somewhat edematous, although in one case this was postulated to arise from right heart failure. Autopsy of rhesus monkeys which died immediately after TBI with 3,000 cGy disclosed congestion of the meninges, with no cerebral edema, and no microscopic changes in the brain (Wilson, 1960). Warren has suggested that it is problematical whether cellular changes seen in the brain after irradiation arise from direct effects, or are secondary to vascular changes (Warren, 1943). In any case it may be that early, radiation induced functional changes of the nervous system can develop in the absence of any immediate, obvious microscopic changes. Thorough repetition of the double vagotomy and atropine experiments certainly is needed, and this ought to be followed by neural measurements which do not drastically affect the animal, and which directly link the nervous system with radiation hypotension.

It is evident that radiation hypotension is not clearly understood, and that no effective therapy is available.

Within the last ten years several radiotherapy centers have developed various protocols to attempt to circumvent the prodromal phase of acute radiation sickness. One approach has been to subject patients to several smaller doses given separately, instead of applying a relatively large single dose (Urtasun, 1983). Dose fractionation has markedly reduced the incidence and severity of the early effects of radiation exposure (Urtasun et al, 1985).[6] However, it is a point of contention whether a fractionated dose is less effective than a single dose in terms of tumor response (Urtasun et al, 1985).[7] Pretreatment with corticosteroids and antiemetics has also very much diminished the severity of the early effects of UHBI (Salazar et al, 1978; Urtasun et al, 1985).[8] Although dose fractionation and premedication very much reduced the incidence and severity of post-UHBI nausea and vomiting, and likely eliminated or reduced post-irradiation hypotension, it is not known that these treatments in fact did affect the blood pressure decline.[9] Also, the drugs have been given prior to irradiation--- if they are not effective when given after exposure this renders their use on irradiation accident victims relatively unattractive. Also, it may not be known whether corticosteroid and antiemetic premedication would be effective in individuals exposed to doses well above 800 cGy.

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[6] Personal communication, Dr. R. C. Urtasun, Department of Radiation Oncology, W. W. Cross Cancer Institute, Edmonton, Alberta.

[7] Personal communication, Dr. R. C. Urtasun.

[8] Personal communication, Dr. R. C. Urtasun.

[9] Personal communication, Dr. R. C. Urtasun.

The lack of insight into radiation hypotension may partly result from the fact that the preponderance of research in radiation biology has, during the last few decades, revolved around cancer radiotherapy, and has also tended to focus on tissue effects which appear weeks to months after exposure, such as hematopoetic aplasia, rather than on changes occurring within the first 48 hours. Consequently, the topic of radiation hypotension has been neglected. Not only might this represent a serious deficiency of information in terms of early irradiation mortality, but also it is possible that early events may influence the course of late tissue effects. Secondly, much of the work on the early cardiovascular effects of radiation exposure is narrow in scope--- individual papers and series of papers have been limited to one hypothesis, and have often involved an incompletely described animal system. Painter's 1946 paper is a stimulating, preliminary foray, but the basic cardiovascular response to whole body irradiation was produced in only 30 rabbits, and important physiological parameters such as the electrocardiogram and the heart rate, were not measured. Her work is virtually the only attempt at exploring each of the three major mechanisms proposed for irradiation hypotension (the hypotheses described in the present review). However, in several of these experiments, too few rabbits were used; therefore at least some ambiguity in the results arising from individual variation is inevitable. Also, Painter and her co-workers appear to have expended limited effort on some rather difficult physiological procedures--- this makes it unlikely that the experiments were performed in an optimal and consistent manner. Radiation hypotension in the rhesus monkey is well described by the combined results of several papers. But, most of the work was oriented around the histamine question, and has neither provided a satisfactory explanation nor an effective treatment, for radiation hypotension.

After consideration of the faults listed in the foregoing, the present study was designed to focus on three main objectives. The first goal was to develop a practical, thoroughly characterized animal model for radiation hypotension. It was intended that this description of the early response to whole body irradiation be considerably superior to previous reports in the radiation hypotension literature in terms of the number of animals irradiated, in terms of the number of physiological parameters recorded in addition to blood pressure, and in terms of the depth and clarity of the quantitative analysis of the data. The rabbit was chosen as the experimental subject because it exhibits an early, and marked cardiovascular response to whole body irradiation, and because it is relatively inexpensive and is easy to handle. The second major objective was to use this rabbit model to systematically search for the mechanism(s) responsible for the blood pressure decline. It was deemed essential to explore several hypotheses, and to incorporate some approaches and techniques which had not been used by previous workers in this field, in order to facilitate an advance past the current body of information. The third aim was to test, in the rabbit, potential treatments for radiation hypotension. The rationales for each of these therapies were to logically derive from the findings of this project, and also to stem from analyses of the radiation biology literature.

II. Materials and Methods

A. General Experimental Protocol and Methods

The recording of arterial blood pressure in irradiated rabbits was central to this study, and two completely different measurement techniques were used. The primary approach was direct and invasive, and involved starving the rabbits overnight, placing the animals under general anesthesia using an inhalation anesthetic, inserting a saline filled catheter in the dorsal aorta. The animals were allowed to recover in a draft-free, heated post-operative enclosure, and upon regaining consciousness were supplied with a carrot, hay, and water. Also, a lucite collar, 1.0 to 1.5 mm thick and 5.4 cm wide, was secured so that the rabbit could not worry its surgical sites, or extract the catheter and/or any other implanted material(s). After about 4 hours, the rabbits were taken to the laboratory for physiological monitoring; in virtually all cases the blood pressure was recorded the day of surgery, with the animals fully conscious. The first 24 rabbits in this study which had been cannulated in the dorsal aorta were taken to the University of Alberta Surgical Medical Research Institute (SMRI), for blood pressure recording. After the 24th experiment, new measurement and recording equipment was obtained and located in the University of Alberta Radiation Research Centre (RRC), and all further work was performed in this facility. At the SMRI, rabbits were placed in a lucite restrainer

(Plas Labs, Lansing Michigan), while all cannulated rabbits monitored at the RRC were secured in a metal restraining box which had a wire cage-type lid. For all invasive blood pressure measurements, the aortic catheter was connected to a pressure transducer which communicated with a strain gauge amplifier, and the resultant blood pressure waveform signal was conveyed to a strip chart pen recorder. A mercury manometer was used to calibrate the blood pressure measurement equipment prior to recording, and in many experiments at the conclusion of recording as well. Electrocardiographic (ECG), heart rate, and ear and rectal temperature measurements were at various stages in the study incorporated as standard practice--- but only in rabbits taken to the RRC. ECG waveform photographs were taken from an oscilloscope trace before and immediately after irradiation, during any major change in aortic blood pressure, at the end of the recording session, and just after any drug injections. The heart rate, and ear and rectal temperatures were retained on the chart recordings as continuous single line tracings. After a baseline physiological recording was established, the animal was transported to the RRC and subjected to TBI in an Atomic Energy of Canada (AEC) Gammacell 220 Co^{60} irradiation unit, or was taken to the W. W. Cross Cancer Institute (CCI) near the University of Alberta campus, and was irradiated variously with either a Siemens 6 MeV linear accelerator X-ray machine, or an Atomic Energy of Canada Ltd (AEC) Co^{60} therapy machine (Theratron 80 or 780). SMRI rabbits were irradiated at the RRC only. After exposure, the rabbit was returned to the SMRI or RRC, and blood pressure recording was resumed. The time taken to reconnect the rabbits for physiological monitoring at the SMRI after irradiation at the RRC, averaged 20.9 minutes from the onset of exposure, with a range of 12-34 minutes, a standard deviation (SD) of 8.03. This was tabulated for 15 SMRI rabbits. in one case it took 57 minutes for recording to resume. For rabbits irradiated and monitored at the RRC, recording was resumed at about 7.1 minutes after the beginning of exposure (range: 1.4-27.5 minutes, SD= 4.78, n=62). When irradiation was performed at the CCI and monitoring took place at the RRC, recording was resumed at about 28 minutes after the beginning of 6 MeV x-irradiation (range: 18.3-43.5 minutes, SD 5.08, n=46). With rabbits that were irradiated with the Theratron 80 Co^{60} machine, it took roughly 36 minutes for reconnection (range: 18.5-55.6, SD 11.8, n=12). If the rabbits experienced a characteristic abrupt fall in blood pressure, which typically occurred at about one hour following exposure, they were monitored for roughly 96 minutes after irradiation. For those animals which did not experience a rapid blood pressure drop, monitoring lasted for up to two hours. Physical and behavioral alterations such as miosis, micturition, struggling, dyspnea, loss of abdominal muscle tone, and a lack of responsiveness to external stimuli were noted on the chart recording. In the early part of this study 47 cannulated, irradiated rabbits were returned to the post-operative enclosure following physiological monitoring. These animals were maintained on regular food and water supplies, and their post-irradiation survival time noted. All the rest of the cannulated animals were killed with sodium pentobarbital at the conclusion of post-irradiation recording, and for some of these animals, the thoracic and abdominal cavities were examined.

The second blood pressure measurement technique was non-surgical and non-invasive, and comprised a modified sphygmomanometer method used on the shaved tail. The rabbits were taken to the RRC and secured in a lucite restrainer (Plas Labs, Lansing, Michigan). Only the systolic blood pressure was measured, using an inflatable tail cuff, an air pressure transducer, and a pulse detector, and the information was recorded by a strip chart pen recorder. The ECG and ear temperature were recorded in the same manner as for cannulated animals. The rabbits were subjected to TBI with an AEC Gammacell 220 at the RRC, or by a Siemens 6 MeV X-ray machine at the CCI. The average time to reconnect the animals for cuff recording was 16.2 minutes. The occurrence of acute hypotension, which usually appeared at about one hour post-exposure, was identified by certain changes in the pulse signal, ECG and ear temperature, and by the ancillary physical and behavioral alterations listed for cannulated animals. Post-irradiation physiological recording periods for rabbits experiencing the sudden hypotension averaged 79.9 minutes (SE=2.8, n=22), while animals not exhibiting this response were monitored for up to 118 minutes (SE=4.3, n=20). Each rabbit in the tail cuff group was killed with sodium pentobarbital.

B. Rabbits Used

The 647 rabbits used for this study belonged to the Dutch Belted breed, and ranged in age from 50 to 935 days. The animals were weaned at approximately eight weeks of age, and were obtained from a closed colony which had been maintained for about 8 years at the time this project commenced, by the University of Alberta Biological Sciences Animal Services. The rabbits were not vaccinated, and those individuals manifesting serious abnormalities were culled from the colony. On campus the animals were also cared for by Biosciences Animal Services, and were caged separately in an air conditioned room which provided about twenty air changes per hour, and a photoperiod of twelve hours. The daily ration comprised one cup of Master Feeds Baby Rabbit Pellets (10% protein), water ad libitum, and a supplement of carrots and hay. Late in the program the maintenance diet was switched to Ralston Purina complete Blend Rabbit Chow (14% crude protein). On a weekly basis the rabbits had their eyes, ears, teeth, and paws inspected and were examined for any lumps. The toenails and if needed, the incisor teeth were trimmed periodically. Animals that were ill or which exhibited gross behavioral abnormalities were not used.

C. Irradiation

Total Body Irradiation - TBI

Three different devices were used to perform total body irradiation. Initially, an Atomic Energy of Canada (AEC) Gammacell 220 Co^{60} irradiation unit was employed to whole body irradiate rabbits weighing less than approximately 1.5 Kg. This unit is located in the RRC at the University of Alberta, and comprises an eight thousand pound lead casing which envelops a cylindrical cage constructed of hollow steel rods containing slugs of Co^{60}. A cylindrical drawer containing the 20.7 by 15.2 cm water-cooled sample chamber is mechanically lowered within the radioactive cage, thus irradiating the sample. Gamma ray dose rates produced by the decaying Co^{60} were measured biannually by the staff of the RRC, utilizing Fricke (ferrous sulphate) dosimetry. With the measured dose rates a time versus dose rate graph was constructed, and the exposure time necessary to provide a particular level of irradiation was calculated using values interpolated from the plot. A substantial portion of the dose-rate line in this graph was extrapolated, resulting in a slowly changing semisystematic error of maximally about 5%. When the drawer was in the irradiating position, the radiation field strength within the sample chamber declined by 20% from the centre to the top and to the bottom, and intensifi[illegible] by 20% from the centre to the chamber wall.

Due to sample chamber space limitations only weanling to subadult rabbits were irradiated using the Gammacell. The animals were secured in canvas bags, placed within the sample chamber, and irradiated for a predetermined time interval to yield nominal total body exposures between 5088 and 628.8 centi-Gray (cGy) at dose rates ranging from 1728 to 691.2 cGy/minute. Exposure length was controlled by an automatic timer.

The second radiation source was a Siemens 6 MeV linear accelerator located at the CCI.[10] This machine uses microwave techniques to accelerate electrons into a gold (Au) target, thereby producing high energy X-rays. The X-rays are delivered in 2 microsecond pulses, which occur every 3.33 milliseconds. Each of these gross pulses has a microstructure of pulses occurring at a frequency of 2.998 GHz. The peak dose rate of each 2.998 GHz micropulse is approximately 3,330 Gy/minute, assuming a 100% duty cycle. The machine operates for 600 microseconds per second, and during the active period the dose rate is 300 cGy/minute at 100 cm from the gold target. However, the nominal average dose over an entire minute is 200 cGy, at 100 cm. For irradiation, weanling and subadult rabbits were placed inside a box fabricated of 0.6 cm thick lucite, and which on its exterior was 24.2 cm long, 16.5 cm wide, and 13.3 cm high (Plate 1). Each flank of the rabbit, at a point roughly lateral to the heart, was approximately 4.5 cm from the facing side of the lucite container. Somewhat larger boxes were used for adult rabbits. The animals were packed in with rice to immobilize them and to overcome dose inhomogeneities due to body contour. Each animal was exposed to a total dose of 1200 cGy, in a horizontal beam. The dose rate with small rabbits was 200 cGy/min~~ute~~, and for adults was 91.2 cGy/minute. A few small rabbits were exposed at 91.2 cGy/minute. For small rabbits the gold target to animal midpoint distance was 100 cm, and the surface field size was 14 (or 15) cm by 26 cm. With all the animals, one half the total dose was directed at one flank, the box rotated 180 degrees, and the exposure repeated to yield the total dose. All the requisite calculations were performed by Dr J. J. Battista of the CCI Department of Physics. The dose calibration was conducted by CCI Physics Department staff using a Capintec 192A dose/dose rate meter which constituted the secondary substandard. This instrument provides accuracy to 0.5%, and possesses a 0.6 cc ion chamber probe, which was placed longitudinally in the center of a rice-filled lucite box and irradiated at 100 cm from the X-ray source. The Capintec 192A was calibrated with a Victoreen 740 ion chamber, the primary substandard, which in turn was calibrated against a National Research council of Canada (Ottawa) ferrous sulphate (Fricke) primary standard in a standard free air chamber.

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[10] Total body irradiation and selective irradiation and shielding procedures were conducted at the CCI with the kind permission and assistance of Dr. J. W. Scrimger of the CCI Physics Department.

The third device employed for total body irradiation was an Atomic Energy of Canada (AEC) Ltd Co^{60} therapy machine (Theratron 80) located at the CCI. Rabbits were placed inside a lucite box and packed in with rice. The animals were then exposed to 600 cGy of gamma rays directed at one flank in a horizontal beam, the box rotated 180 degrees, and the irradiation completed to provide a total dose of 1200 cGy. The Co^{60} source to rabbit midpoint distance was 80 cm, and the surface field size was 15 cm by 26 cm. Dose rates ranged from between 120 to 91 cGy/minute. Exposure lengths, source to target distance, and dose rates were calculated by staff of the CCI Physics Department. At the beginning of each month the Co^{60} dose output was calculated for the 15th day of the month. The resultant dose rate value was used to calculate source to target distances and exposure times throughout the entire month. Dose rates thus determined were 0.5% low on the first of each month, and 0.5% high on the last day of every month. The Co^{60} source output was checked regularly by CCI physics personnel using the same instrumentation and methods as with the Siemens 6 MeV linear accelerator. Late in the project the Co^{60} therapy unit was replaced by a very similar, but new machine (Theratron 780) from the same manufacturer (AEC). Irradiation and dose determination protocols were not changed following this replacement.

Sham Irradiation

Ten rabbits ranging in age between 57 and 92 days were starved overnight, had a saline-filled catheter implanted in the dorsal aorta, and had 4 electrocardiographic electrodes attached, all according to the methods described in Materials and Methods D. After a minimum 4 hour post-operative recovery period, the animal was taken to the RRC and the aortic blood pressure, heart rate, electrocardiogram, and ear and rectal temperatures were monitored. The rabbit was secured within a canvas bag and placed in the sample chamber of an AEC Gammacell 220 The animal remained within the sample chamber for 70 seconds, during which time the Gammacell was not operated in the irradiating position. Physiological monitoring was resumed, and continued for two hours following sham irradiation. At the conclusion of the recording period each rabbit was killed by injection with 1 to several ml of concentrated (240 mg/ml) sodium pentobarbital (Euthanyl, MTC Pharmaceuticals, Hamilton, Canada).

Selective Irradiation/Shielding''

Irradiation/Shielding of the Heart Region

The rabbits were transported to the CCI, placed

''Note: Irradiation of the liver with a portion of the gut, and irradiation of the upper half body are described in Appendix III.

within lucite boxes, and securely packed in with rice. The lucite box was placed on the bed of a radiotherapy treatment simulator (Picker, or Phillips) and the internal anatomy of the rabbit visualized fluoroscopically. The heart region was circumscribed by adjustable horizontal and vertical grid lines on the fluoroscope screen (Plate 2). This produced corresponding changes in a field defining light beam which was projected onto the side of the rabbit box; the illuminated 4 by 4 cm square was then demarcated using a grease pencil. The simulated field was then marked on the contralateral side of the rabbit container. The heart region included the heart and adjacent great vessels, and those elements of the lungs close to and surrounding the heart. During every use of the treatment simulator an X-radiograph was taken which recorded exactly the image presented on the fluoroscope screen, including field defining grid lines (Plate 2). Fluoroscopic examination of each rabbit lasted less than about 2 minutes, and according to Dr S. R. Usiskin, head of the CCI Physics Department, the fluoroscope would not generate more than 3 cGy/minute. In early heart irradiation tests, an X-radiograph of the animal was taken at the SMRI prior to selective irradiation at CCI. The rabbit was placed within one of the previously described lucite boxes, packed in with rice, and an X-radiograph produced. The animal was later transported to the CCI and placed in a lucite box with rice packing. The heart was then located by examining the SMRI X-radiograph; an approximately positioned 4 by 4 cm or 5 by 5 cm square area was then marked on the rabbit box. This method was used on the first three heart irradiated rabbits, while with the fourth animal the accuracy of this approach was evaluated using fluoroscopy. All further selective heart irradiated animals were prepared using fluoroscopy, as were all the heart shielded rabbits.

After the heart was located and the lucite box marked, the rabbit was placed on the treatment bed of either the Siemens 6 MeV X-ray machine or the AEC Co^{60} therapy unit. For selective irradiation, a field defining light beam was adjusted and aligned to match the marked area on the lucite box. This procedure concurrently adjusted the irradiation unit so that an x- or gamma-ray beam collimated to 4 by 4 cm square, would be directed at the heart region. With the 6 MeV linear accelerator the dose rate was 200 cGy/minute, to yield heart area exposures of either 1200 or 2000 cGy. The Co^{60} unit supplied dose rates of either 67.2 or 66.6 cGy/minute, for a heart region exposure of 1200 cGy. With both devices one half the total dose was delivered to one side with a horizontal beam, the box rotated 180 degrees, the exposure field repositioned, and the irradiation completed. Also, with both radiation sources, the slightly lower dose rates resulting from the relatively small field size, were corrected for by increasing the length of exposure.

In heart shielding experiments, a block of "Cerrobend" 4 cm wide, 4 cm high, and 7 cm long, was aligned with and placed against the marked area on the rabbit box (Plate 1). Cerrobend (Canada Metal Co.) comprises 26.7% lead, 50.0% bismuth, 13.3% tin, and 10% cadmium, and presents a half value layer of approximately 14 mm to 6 MeV X-rays, and about 12 mm to Co^{60} gamma rays. A second such block was placed end to end with the first to attain a combined length of about 14 cm. Each block also came fitted with a 0.5 cm steel plate at one end, and thus additional shielding was provided by 1 cm of steel. The rabbits were subjected to TBI with the Siemens 6 MeV X-ray unit, which provided a dose rate of 200 cGy/min for a total exposure of 1200 cGy, or with an AEC Co^{60} therapy machine which delivered dose rates ranging between 89.8 and 91.2 cGy/min, for a total dose of 1200 cGy. With both devices one half the total dose was delivered from one side with a horizontal beam, the rabbit box rotated 180 degrees, the shielding blocks repositioned, and the irradiation completed. Calculations performed by Dr S. R. Usiskin of the CCI Physics Department, indicated that during whole body exposure of rabbits with 6 MeV X-rays, the 14 cm of Cerrobend shielding would have reduced the heart region dose to less than 1.2 cGy. The dose to the shielded area would have been even less during exposure to Co^{60} gamma rays, and with both irradiation sources, the amount of radiation would have been further reduced by the 1 cm of steel.

Irradiation of the Head

Two approaches were used for selective irradiation of the head, and 3 rabbits were subjected to this kind of exposure. In one case the animal was packed into a lucite box with rice. Locating the lower portion of the head within the rice was accomplished fluoroscopically using a radiotherapy treatment simulator (Picker, or Phillips). A rectangular area which measured 6 cm vertically and 5.5 cm horizontally, and which corresponded to the area presented by the side of the rabbits head was then marked on the box. With the other two rabbits fluoroscopy was not performed, and the position of the lower portion of the head was estimated. For these two irradiations, plastic bags were filled with water until they were 5 to 10 cm thick and then placed on each side of the head. The water bags were intended to overcome inhomogeneities in dose deposition resulting from variation in the contour of the head. All 3 rabbits received 1200 cGy to the head at a dose rate of either 130 or 129 cGy/minute delivered by a Theratron 780 Co^{60} therapy unit, employing a horizontal beam collimated to 6 by 5.5 cm. One half the total dose was delivered to one side, the box rotated 180 degrees, the exposure field realigned, and the irradiation completed.

D. Physiological Measurement Techniques

Blood Pressure Measurement and Recording

Catheterization of the Dorsal Aorta for Direct Arterial Pressure

Rabbits were supplied only water for about 16 hours prior to surgery, and on the morning of the operation were anesthetized with 5% halothane gas (Fluothane, Ayerst) in 95% oxygen, administered through a face mask. The anesthetic was delivered by a small animal anesthetic machine (Fraser Sweatman) operating in a circle-type rebreathing arrangment. After attaining a surgical level of anesthesia, about 4 square cm of fur was shaved from between the shoulder blades, and at least 6 square cm from the left femoral area. The rabbit was supinated and the left hind leg was tied in a laterally extended position. After swabbing the femoral area with 70% ethanol, a 1 to 2 cm long skin incision was made at right angles to the femoral artery. Using a combination of scissor cutting and blunt dissection an approximately 1 cm length of the femoral artery was freed from the femoral nerve and vein, and cleaned of adhering connective tissue. Two strands of 4-0 or 3-0 silk were placed around the artery, saline moistened gauze placed over the surgical site, and the animal positioned on its right side. The shaved dorsal area was swabbed with 70% ethanol, and a roughly 0.5 to 2 cm skin incision made. A metal rod 31.9 cm long and 2.36 mm thick, which at one end was flattened and drilled through to form an eyelet, was burrowed beneath the skin of the left flank until the eyelet protruded through the femoral incision. A few cm of a polyethylene tube at least 30 cm long was looped through the steel rod eyelet, and the rod together with tubing was drawn anteriorly below the skin of the left flank and out the dorsal incision. Thus the tubing was implanted below the skin of the flank between the femoral and dorsal sites, and also protruded from each incision. The animal was supinated, the left hind leg extended, and the femoral artery tied off distally with one of the two previously placed silk strands. The polyethylene tubing was filled with heparinized 0.9% saline and the end extending from the dorsal incision clamped. The heparinized saline was prepared well before the operation by dissolving 0.9 gm of NaCl in 100 ml deionized sterile water, adding powdered sodium heparin (Grade 1 from porcine intestinal mucosa, Sigma, St. Louis, Mo.), and sterile filtering the solution. Gentle traction was then applied proximally and distally on the femoral artery using both the tied and untied silk threads, and a small area on top of the vessel midway between the ligatures was stripped of outer wall layers. The artery was incised at this site, and the catheter slipped in and advanced anteriorly. In early operations the cannula tip resided in the upper reaches of the femoral artery, but for the majority of catheterizations it rested at some point along the lower third of the dorsal aorta. For rabbits weighing roughly between 800 and 1400 gm PE-50 (ID 0.58 mm) polyethylene tubing (Clay Adams, NY) was implanted, while animals weighing more than about 1400 gm could accomodate, and often were implanted with PE-90 (ID 0.86 mm) tubing.

After installation the catheter was unclamped, allowing blood to flow out the end so that any trapped air or incipient thrombi would be expelled. If blood appeared very slowly, the catheter was partly or completely withdrawn and then readvanced. After being flushed with at least 0.4 ml of heparinized saline, the tubing was sealed at the free end with heat or by tying it into 3 closely spaced knots. The catheter was then secured to the femoral artery by tying the two together with lengths of surgical silk, and the femoral incision was closed with interrupted stitches of 3-0 catgut (Chromic, Ethicon). The dorsal incision was sutured with interrupted stitches of 3-0 or 2-0 surgical silk, and both this and the femoral site were swabbed with 2.5% iodine solution. The external segment of the catheter was coiled and taped to the animals back.

In some rabbits which were to be tested with pharmacologic or chemical agents, the inferior vena cava (IVC) was cannulated via the femoral vein. When included, this procedure was usually performed just prior to aortic cannulation so that the thin-walled femoral vein would not collapse due to absence of blood flow. Skin incisions were made in the dorsal and femoral areas, and the femoral vein and artery freed. The venous cannula (polyethylene PE-50) was drawn along beneath the skin of the left flank together with the arterial catheter. This was accomplished using the same method described previously for the arterial catheter alone. The IVC catheter was filled with heparinized saline and clamped at the end extending from the dorsal incision. Gentle traction was applied to the femoral vein both distally and proximally with two strands of 3-0 silk, and the vessel stripped of any adhering tissue. The vein was incised midway between the two traction points and the PE-50 catheter, with the tip bevelled, was inserted. The catheter was advanced until its tip resided anywhere along the upper half of the IVC. Blood was withdrawn, the catheter was flushed with a few ml of heparinized saline, the free end was clamped, and the femoral vein was tied off distally with 3-0 silk. The femoral vein and catheter were tied together proximally with 3-0 silk, and the free end of the catheter was sealed by tying it into 3 closely spaced knots. The femoral artery was then cannulated, skin incisions sutured, and the external portions of the venous and arterial catheters were taped to the animal's back.

External Transducer Apparatus for Direct Arterial Pressure

A Gould-Statham P23ID pressure transducer with a clear plastic dome enclosing a strain gauge diaphragm was used to directly measure aortic blood pressure. The transducer was clamped to a metal stand at a height which was level with the rabbit's heart, while the animal was secured within a metal restraining box (Figure 2). The transducer dome included two ports; one directed forward to receive blood pressure wavefronts, and the second opening laterally to accept calibration pressures. Three way stop-cocks were fixed to each dome port, and the forward port stop-cock was fitted with a luer needle. Initially a 23 gauge (nominal ID 0.0125 ", OD 0.025") needle was employed but this was later changed to 18 gauge (nominal ID 0.033", OD 0.050"). The aortic catheter was fixed to the 23 gauge needle to connect the rabbit's arterial system with the pressure transducer. However, with an 18 gauge needle, short lengths of different diameter polyethylene tubes were slipped over each other to create a step-down connection, which was slid over the needle, and into which was inserted the rabbit cannula. Affixed to the forward stop-cock vertical opening was a syringe containing heparinized 0.9% saline (150 U/ml of sodium heparin, from porcine intestinal mucosa, Sigma, St Louis, Mo), for flushing the rabbit catheter. Prior to connection with the needle, the cannula was cut open and a brief outflow of blood permitted in order to expel any incipient thrombi. The forward port stop-cock was then adjusted, heparinized saline flushed through the needle, and the cannula connected during the flush. The catheter was flushed with the heparinized saline well beyond the time at which all signs of blood were cleared. The lateral dome stop-cock was then completely closed, the forward stop-cock opened so that the rabbit cannula could communicate with the transducer, and blood pressure recording proceeded.

Fixed to the lateral port stop-cock was a water filled, 4/16" (3.2 mm) OD tygon tube, which in turn was connected to a short length of 3/8" (7.9 mm) OD tygon tubing. The large diameter tubing was fitted to the bottom outlet of a 500 ml glass reservoir (aspirator bottle, Kimble, USA). The reservoir contained about 200 ml of deionized water, and had its top opening plugged with a rubber stopper. A cylindrical, hollow plastic tubing connector went completely through the stopper, and its external end was fixed to 4/16" (3.2 mm) OD tygon tubing. This tubing then ran to a plastic "T" connector. Two 4/16" (3.2 mm) OD tygon lines were slipped onto the two free T connector limbs: one tube travelled to a mecury manometer (Baum Co., New York, NY) which measured pressures from 0 to 200 mm Hg; and the second to a length of 0.9 cm OD rubber tubing, which communicated with a two way valve, a pin valve, and a 3.7 cm OD rubber bulb.

The pressure transducer output was fed to a strain gauge amplifier (Beckman, or Gould), and was then traced by a chart recorder pen (Beckman, or Gould) onto a paper strip. Very early in this project a Beckman type R multi-channel chart recorder which produced a curvilinear pen sweep was used. After the 24th cannulated rabbit blood pressure recording, this was replaced by a Beckman R411 4 channel chart recorder with rectilinear pen action. The Beckman R411 was replaced about midway through the study by a Gould Instruments 2400s recorder, offering a pressurized ink system and rectilinear pen movement. For invasive blood pressure measurement, the recorders were usually operated with a chart paper feed rate of 5 mm/min.

Calibration and Testing of the External Transducer System

Static pressure calibration was always performed prior to recording rabbit blood pressure. For the majority of recordings, and nearly all those done in the second half of the study, static calibration was also conducted at the conclusion of pressure recording. However, prior to calibration and rabbit catheter connection, the transducer dome and all fluid filled lines were inspected for air bubbles. Then the system pressure was raised by repeatedly squeezing the rubber bulb, and pressure maintained by closing the two way valve. The transducer dome stop-cocks were then opened so that water flowed into the dome and out through the connecting needle. Lines were rapped at various points to liberate trapped air bubbles. The forward transducer stop-cock was closed and the system pressure released. Pressure calibration was then performed. The forward transducer stop-cock was adjusted so that the forward port was completely sealed off, and the side port stop-cock was left open to the reservoir tube. The chart recorder pen was positioned on the zero pressure line with the entire measurement system open to the atmosphere. The manometer, reservoir plumbing, and transducer dome contents were then pressurized to either 150, 160 or 200 mm Hg, by pumping the rubber bulb after setting the pin valve for one-directional airflow. Once the desired maximum pressure was attained, the two-way valve was quickly closed and the recorder pen adjusted on the topmost chart line. The system was reopened to atmosphere, and the chart recorder zero rechecked. Blood pressure recording could then proceed.

The response accuracy over a range of pressures, or linearity, was periodically examined for the P23 ID transducer by supplying 3 pressures, maximum, one-half of maximum, and zero. The resultant pen trace positions with respect to the appropriate chart paper lines indicated the level of linearity. Linearity was more precisely evaluated using an on-line computer, as described in Materials and Methods D.

The measurement stability over time of the entire system was assessed by comparing calibration tracings produced before and after 1 to 2 hour long rabbit experiments. The zero pressure tracings taken with the transducer open to atmosphere were compared, as were the tracings resulting from full scale pressure (160 or 200 mm Hg). The extent of system drift was evaluated on the basis of any discrepancy between the pre-experiment and post-experiment tracings. This assessment and system calibration were not performed at the end of every recording session.

For dynamic system response testing, the pressure transducer was connected via fluid filled tubing to an electrically operated pressure wave apparatus. This device comprised a loudspeaker 14 cm diameter, a 3 cm diameter plastic disc glued to the center of the loudspeaker cone, and a stainless steel bellows 0.6 cm diameter and 2.6 cm long which was cemented to the plastic disc (Figure 3). The bellows connected to 4.3 cm long steel tube, which was fitted at the open end with a two way stop-cock. A wire lead ran from the speaker coil to an electronic wave function generator (3310A Function Generator, Hewlett Packard). This instrument is able to produce square wave signals at frequencies ranging from

0.0001 Hz to 100 kHz. The loudspeaker and bellows translated the electric signal to a square pressure wave which travelled through the connecting catheter, transducer needle, and three-way stop-cock, to the transducer diaphragm. The resultant transducer output was displayed on, and photographed from a storage oscilloscope (7633, Tektronix). The response time of the transducer was then determined by measuring the pressure squarewave rise time on the oscilloscope photographs. The effects of various diameters, lengths, and types of catheter materials, and of different connecting needle sizes, on transducer response were measured using this method. Dynamic response testing with this apparatus was performed early in the study, while a simple check of dynamic performance was routinely conducted by reading the systolic to diastolic pressure difference from the chart recording. A blood pressure waveform amplitude equal to or greater than roughly 25 mm Hg was accepted as satisfactory.

The level of accuracy and waveform detail provided by the external transducer-fluid filled catheter system was also evaluated by comparing blood pressure waveform results with those obtained using an indwelling catheter transducer. Rabbits heavier than approximately 1.7 kg could accomodate a catheter possessing a pressure diaphragm near the tip (average OD 1.33 mm, Mikro-Tip Catheter Pressure Transducer, Millar Instruments Inc., Houston, Texas.). This catheter was drawn beneath the skin and installed in the dorsal aorta via the left femoral artery using the same methods described earlier for fluid filled catheters. The only exception was that flushing with heparinized saline was omitted. The catheter transducer was plugged into a preamplifier (TCB-100 Transducer control Unit, Millar) which was connected to an amplifier (Beckman, or Gould), and the transducer output was written out by a chart recorder (Beckman, or Gould) onto a paper strip. At the conclusion of each experiment the rabbit was killed by injection with Euthanyl. and the transducer catheter removed. The transducer was soaked in warm soapy water, and the tip gently cleaned with wet gauze.

Static pressure calibration of the catheter transducer was performed just prior to each blood pressure recording. The static calibration had to be effected electronically by switching the relevant dial settings on the TCB-100 control unit. This sent electrical signals to the recorder which traced a series of pressure levels on the chart paper. For dynamic response testing of the catheter transducer, a transducer guard was connected to the loudspeaker-bellows pressure waveform apparatus. The transducer guard comprised a plastic cylinder that from its midpoint tapered to one end: this narrow end was equipped with a silicone rubber nipple, while the non-tapered half terminated in a male luer fitting (Figure 6 b). The transducer tip was inserted into the plastic cylinder through the nipple, and dynamic response was measured in the same manner as for the Gould P23 ID external transducer.

Noninvasive Blood Pressure Measurement

This method was used to measure systolic aortic pressures, and did not involve general anesthesia or surgery. The rabbit's tail was shaved using fine-bladed electric clippers, and the animal was secured in a plexiglass restrainer (Plas Labs, Lansing, Michigan) which held the neck and restricted body movement. Rolled cloth bandages were packed alongside the rabbit, and a cuff placed around the base of the tail (Figures 4 and 5). The cuff comprised a 1.3 cm section of copper pipe (14 mm ID, 15.9 mm OD) surrounding an inflatable internal sleeve of thin walled latex. The cuff was fabricated by first drilling a hole into the copper pipe, and working a 2.45 cm long section of narrow copper tubing (ID 1.51 mm, 3.2 mm OD) into the drillhole. The end of the smaller tubing was set flush with the interior surface of the copper pipe and soldered in place.

The latex sleeve was longer than the copper cylinder, and both protruding ends of the latex were reflected back over the copper pipe. These flaps were gripped in place by a cylinder of 3/4" heat shrinkable tubing (Alpha Wire Corporation, Elizabeth, New Jersey), which when heated, contracted tightly around the copper pipe.

A piezoelectric transducer (Cardio-microphone Model 1010, Biocom Inc.) was placed on the tail just behind the cuff, and was connected via turntable tone arm cable, to an indirect blood pressure coupler/amplifier (Type 9863A, Beckman Instruments). A wrapping of woven adhesive bandage (Elastoplast, Smith and Nephew Inc., Lachine, Quebec) trimmed to about 5 by 5 cm, held the transducer in place and maintained light pressure between the transducer and tail. Flexible, thin walled plastic tubing (ID 3 mm) was fixed to the slender copper tube emanating from the cuff, and then communicated with a length of tygon tubing (ID 4.6 mm) which was slipped over the centre limb of a plastic "T" connector. Tygon tubes were slid over the T connector side arms, and one line went to an electrically operated air pressure pump (Cuff Pump, Narco Biosystems Inc., Houston, Texas), while the other line ran to the indirect blood pressure coupler. The air pump could automatically cycle at preset intervals of 0.5, 1.0 or 2.0 minutes, or could be actuated manually as desired. The tail transducer and indirect blood pressure coupler could be operated together in either of two modes: (1) pulse detection, in which low frequency mechanical displacment of the transducer diaphragm generated pulse signals; or (2) detection of Korotkoff sounds which are of high frequency and also produce transducer diaphragm displacement. The former was always used, so that modulation in tail size associated with pulsatile systolic blood flow was detected. The indirect blood pressure coupler contained a pressure transducer which measured air pressure in the entire system, and transmitted this information together with the pulse signal to the chart recorder pen drive (Beckman R411). This produced a curve of pulse superimposed on pressure. The chart recorder paper feed was run at either 25 or 50 mm/min. The indirect blood pressure coupler also contained an air pressure manometer and dial indicator. This facilitated calibration of the air pressure transducer output as indicated on the chart recording. The cuff pump was adjusted to produce a pressure of 200 mm Hg.

Cuff inflation to 200 mm Hg blocked tail blood flow rendering the pulse transducer inactive, and producing a smooth line air pressure trace on the chart recording (Plate 3). As air bled from the system at a controlled rate cuff pressure declined to approximate equivalence with the rabbit's systolic pressure, allowing resumption of tail blood flow. This caused the outside diameter of the tail to oscillate and the transducer generated a pulse signal. The rapid pulse oscillations appeared on the air pressure decay curve at a point corresponding to the systolic pressure; diastolic pressure could not be determined with this method.

In two rabbits a fluid filled catheter was implanted in the dorsal aorta, and the blood pressure measured with the external blood pressure transducer and tail cuff simultaneously. Both rabbits were total body irradiated in the Gammacell 220. One animal was exposed to 996 cGy at a dose rate of 1328 cGy/minute , and the other received a TBI dose of 1200 cGy at 1169.6 cGy/minute.

Catheterization of the Inferior Vena Cava for Pressure Measurement

Attempts were made to introduce several different types of catheters, including the Millar catheter tip transducer, fluid filled PE-90 and PE-50 tubing, and an umbilical vessel catheter, into the right ventricle. Fourteen rabbits were tested and right ventricular catheter placement was verified in 2 cases, suspected for 2 others, and definitely not achieved in 10 animals. Because of the poor success rate with the early attempts at right ventricular catheterization, cannulation of the inferior vena cava (IVC) with fluid filled PE-90 or PE-50 tubing, to a level near the venous entry into the right atrium, was attempted in six rabbits. It was reasoned that a rise in right ventricular pressure stemming from enhanced pulmonary vascular resistance might also cause pressure within the IVC to increase appreciably.

Rabbits were starved overnight and anesthetized with halothane. The left femoral area, and a small area of the upper back were shaved with electric clippers. Both sites were incised, and two polyethylene PE-50 catheters were drawn posteriorly under the skin. The femoral vein was cannulated, and the catheter was advanced so that the tip within the IVC nearly entered the heart. Blood was withdrawn and the catheter was flushed with heparinized saline. The portion of the catheter extending from the dorsal incision was tied. The femoral artery was then catheterized, and the wounds were closed. Four ECG electrodes were clamped to the chest and legs, and the animal was allowed to recover in a heated post-operative enclosure.

Catheterization of the Right Ventricle for the Measurement of Right Ventricular Pressure

After the IVC measurement tests were performed (preceding section), catherization of the right ventricle was attempted again, but with fluoroscopy. A 3F umbilical vessel catheter (Argyle) was snaked into the right ventricle via the left femoral vein, under fluoroscopic control. In the same rabbit, a 4F neonatal cardiac balloon tip catheter was introduced into the right ventricle via the right external jugular. Plate 4 is a reproduction of an X-radiograph taken with the fluoroscopy unit, approximating the image produced on the fluoroscope screen, which shows both catheters in this rabbit. However, the neonatal cardiac catheter was used exclusivaly for all subsequent right ventricular catheterizations.[12] After the preliminary test, right ventricular catheterization using fluoroscopy was attempted with 19 rabbits. The method is described below.

Rabbits were starved overnight, transported to the the Surgical Medical Research Institute at the University of Alberta, and anesthetized with halothane. About midway through the right ventricular catheterization project, it became standard practice to inject about 0.24 mg of atropine sulphate (Squibb) intramuscularly when the rabbit was fully anesthetized.[13] Using fine bladed electric clippers fur was shaved from the left femoral region, between the shoulder blades, and the entire ventral half of the neck from the jaw insertion to the clavicles. The dorsal aorta was cannulated with fluid filled polyethylene PE-50 tubing according to the technique described earlier in Materials and Methods. The only difference in cannulating the dorsal aorta of a rabbit belonging to the right ventricular catheterized group, was that the aortic cannula tip was cut to a bevel. An approximately 2 cm lengthwise skin incision was made in the neck, directly above the right external jugular vein. Using blunt dissection the jugular was exposed and cleared of adhering fat and connective tissue. Two lengths of 3-0 silk were passed beneath the vein, and gentle traction applied cranially and caudally so that the vessel was held taut. Outer layers of connective tissue tightly enveloping the vein were stripped away on top of the vessel so that a small very thin walled area was created. This area was incised and tension on the cranial traction thread increased to stem bleeding. A 4 French (OD 1.25 mm) neonatal cardiac balloon catheter with side ports at the tip, was sprayed with silicone lubricant, and was introduced into the vein. At no time was the catheter balloon inflated, and

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[12] The cardiac balloon catheter owing to its relatively greater size and stiffness could be passed into the heart only through the external jugular. This catheter was chosen for right ventricular pressure recording over the umbilical catheter for the reasons described below. The cardiac catheter possessed three small, closely spaced side ports on each side, starting about 0.9 mm from the tip, which would continue to facilitate pressure recording if the catheter tip was jammed against the heart wall. The cardiac catheter also was equipped with a luer connector, which made attachment of flushing devices during implantation simple, and which was easily affixed to the external pressure transducer. In contrast, the umbilical vessel catheter had an open tip which might not supply pressures when pushed into the apex of the ventricle. Also, the connector end was flanged and without any fitting. Hence the cardiac catheter was more practical, and due to its larger diameter, 4F as opposed to 3F for the umbilical catheter, pressure waveforms would be less damped than with the umbilical tubing.

[13] Seven rabbits died during right ventricular catheterization from heart failure; the catheter may have stimulated venous and atrial stretch receptors eliciting considerable vagal outflow, which, combined with the negative chronotropic effect of halothane on the cardiac pacemaker, caused ventricular fibrillation. It thus became standard practice to administer about 0.21 to 0.26 mg/kg atropine sulphate intramuscularly prior to surgery. This would not be expected to affect the cardiovascular response to radiation, since

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[13] (cont'd) the dose of atropine was well below that known to influence the hypotension, as indicated in Figure 45. Also, several hours elapsed between atropine injection and irradiation, so that the drug should have been cleared well before TBI.

it was eventually replaced by a smooth cap of silicone rubber (Self-levelling RTV, Dow Chemical Corp.). A guide wire was slipped into the catheter lumen and advanced to the tip. The leading end of the guide wire was bent prior to insertion into a roughly 3 cm long limb, at a 30 degree angle, so that the catheter would assume this leading end configuration when the guide wire was inserted. The catheter was advanced a predetermined distance down the vein, and temporarily secured when its tip was approximately at heart level. A mobile fluoroscopy unit (Siemens) was positioned above the animal's chest. Under periodic fluoroscopic visualization, the catheter was directed into the apex of the right ventricle. The guide wire was removed, blood withdrawn using a syringe, and 2 to 4 ml of heparinized 0.9% saline (150 U sodium heparin/ml, from porcine intestinal mucosa, Sigma, St Louis, MO) was flushed through the catheter. Then 1 to 2 ml of contrast medium (either 50% or 90% Hypaque, Wintrop) was rapidly injected into the catheter as a single bolus, and the flow of this material through the right heart circulation was observed to verify catheter placement. Heparinized saline was again flushed through and the catheter stop-cock completely closed. Measurements performed by staff from the Occupational Safety Department at the University of Alberta using an air ionization chamber (Victoreen), revealed that the fluoroscopy unit exposed the surgical table top to 250 roentgens per hour. The fluoroscope settings were 85 kv and 2.5 mA. For most right ventricular catheterizations the fluoroscope was operating for about 5 to 15 minutes (in a few cases much less than 5 minutes), and rarely, up to roughly half an hour. Thus most of the rabbits were exposed to between 42 and 62.5 roentgens, and possibly some received doses up to 125 roentgens.

After the catheter was placed, the fat, fascia, and skin of the neck were closed with interrupted stitches, and the catheter was securely tied at several points along the surgical site. The rabbit was rolled into a prone position, and the dorsal incision for the aortic catheter sutured. The aortic and right ventricular catheters were coiled, covered with gauze, and taped to the animal's back. The rabbit was then transported to a post-operative enclosure at Biological Sciences Animal Services on the University of Alberta campus.

Apparatus and Protocol for Measuring Right Ventricular Pressure

The apparatus for right ventricular measurement was identical to that for aortic pressure recording described earlier in Materials and Methods, except that only one type of recorder, a Gould 2400s, was used. A Gould-Statham P23 ID external blood pressure transducer, strain gauge amplifier, and chart recorder were calibrated to from 0 mm Hg to either 50 or 100 mm Hg. The right ventricular catheter stop-cock was opened, about 1 ml of blood withdrawn with a syringe, and several ml of heparinized 0.9% saline (150 U sodium heparin/ml, from porcine intestinal mucosa, Sigma, St Louis, MO) flushed through. Heparinized saline was flushed forward through the transducer stop-cock, and during this flush the right ventricular catheter was connected. The stop-cock was opened to allow blood pressure waves from the catheter to enter the transducer, and recording proceeded.

For aortic pressure measurement, a 23 gauge needle was fixed to the male end of a three-way teflon stop-cock. A syringe containing heparinized saline was slid over the female connector directly opposite the male, and saline flushed through. The stop-cock was then closed off to the syringe. A cylindrical plastic transducer guard possessing a male luer connector at one end, and tapered to a small opening fitted with a silicone rubber nipple at the other end, replaced the syringe (Figures 6a and 6b). The syringe was fitted to the vertical female connector, the plunger depressed, and the stop-cock adjusted so that saline squirted through the transducer guard nipple. During this flush a catheter transducer (Mikro-tip Catheter Pressure Transducer, Millar Instruments Inc.) was advanced through the nipple into the cylinder.

The transducer was plugged into a wheatstone bridge preamplifier (TCB-100 Transducer control Unit, Millar) which was connected to an amplifier (Universal Amplifier, Model 13-4615-55, Gould Instruments Inc.) (Figure 2). The amplifier output was traced by recorder pen (Gould 2400s) onto a paper strip. The aortic catheter was cut open and blood allowed to drain so that any incipient thrombi were expelled. The stop-cock was adjusted, saline flushed through the needle, and the catheter slipped over the needle shaft. The stop-cock was set to allow aortic blood pressure waves to travel from the catheter to the transducer, and recording proceeded. This method of aortic blood pressure measurement was only used for rabbits which were also catheterized in the right ventricle.

The catheter transducer was calibrated prior to and at the termination of blood pressure recording, using similar equipment and methods as for calibration of the Gould P23 ID pressure transducer. However, zero pressure was calibrated by resting the naked transducer tip on a stable surface, and positioning the chart recorder zero with the TCB-100 preamplifier zero adjustment control. The zero level was rechecked at the conclusion of blood pressure recording by again placing the transducer in air. A three-way teflon stop-cock was connected, via a water filled, 4/16" (3.2 mm) OD tygon line, a "T" connector, and a short length of 3/8" (7.9 mm) OD tygon tubing, with the bottom outlet of a water reservoir bottle. The male end of the plastic transducer guard was slipped into the female stop-cock connector which was fixed to the reservoir line. The system was pressurized by pumping the rubber bulb, and then opening the stop-cock to permit water to squirt straight out. During the latter process, the catheter transducer was slid into the plastic guard via the rubber nipple. The pressure was raised to 200 mm Hg and the recorder pen positioned on the top chart line. The pressure was released, the stop-cock closed, and the transducer catheter was connected to the aortic cannula for blood pressure measurement.

The Electrocardiogram and Heart Rate

Attachment of electrocardiographic (ECG) electrodes was routinely performed on rabbits which were to be subjected to arterial blood pressure monitoring with either the invasive or noninvasive methods. Right ventricular catheterized rabbits also were equipped with ECG electrodes. For operated animals the ECG electrodes were attached during general anesthesia and comprised 14 by 3 mm Ni-Ag wound clips (Aesculap, or Propper). One electrode was clamped to each side of the ventral aspect of the chest, and to the medial surface of the right hind leg. About midway through the study, with the arrival of a Gould 2400s chart recorder and

Gould ECG/heart rate amplifier, it became standard practice to also clamp an electrode to the medial aspect of the left hind leg. Each site of attachment was usually washed with 70% ethanol prior to electrode placement. The same general method was used with rabbits that were to be tail cuff monitored, except that electrode attachment was effected while the animals were conscious. Thus to minimize discomfort, fur was shaven from the placement areas and lidocaine hydrochloride solution (Xylocaine 40 mg/ml, Astra Chemicals Ltd, Mississauga, Ont.) was swabbed over the skin.

Two different procedures were used to monitor the ECG; one when the Beckman R411 chart recorder was used for blood pressure measurement, and the second method was applied in conjunction with the Gould 2400s recorder. With the first approach the three ECG electrodes on the rabbit were connected to a differential AC amplifier (Brookdeal 9454, England) via shielded turntable tone arm cables. The leads were clamped to the ECG electrodes with alligator clips. The same lead polarity was maintained between rabbits. The differential amplifier transmitted the voltage information to a storage oscilloscope (7633, Tektronix) which displayed the ECG waveform.

For a few of the experiments in which the Beckman R411 chart recorder was used, a heart rate amplifier (Beckman Instruments) was connected to three ECG leads. The amplifier electronically determined the heart rate in beats per minute on the basis of ECG voltages. The heart rate information was transmitted to the recorder pen drive which traced a single, continuous line on the chart paper.

For the second ECG monitoring method, rabbits were fitted with 4 electrodes. The electrodes were then connected to an ECG/heart rate amplifier (ECG/Biotach, Model 13-4615-65, Gould Instruments) via shielded turntable tone arm cables. The wire leads were connected to the rabbit's ECG electrodes with alligator clips. The Gould ECG/heart rate amplifier permitted various combinations of voltage references between the 4 leads; thus the most informative and cleanest version of the ECG waveform was selected. The ECG/heart rate amplifier electronically determined the heart rate from the ECG voltages, and conveyed this information to the recorder pen drive (Gould) to produce a single line tracing on the chart paper. The heart rate system of the ECG/heart rate amplifier can operate in either of two modes: (1) the heart rate computed instantaneously on the basis of each ECG waveform, and expressed as such, or (2) the heart rate supplied as a running average which is determined on the basis of the ECG waveforms occurring over 5 seconds. The ECG/heart rate amplifier and chart recorder were calibrated prior to each blood pressure recording session. This was accomplished by setting the chart pen at zero, activating the calibration electronics of the amplifier, and adjusting the pen control and/or amplifier. The ECG voltage was also transmitted to a storage oscilloscope (7633, Tektronix).

During all 3 lead and 4 lead ECG monitoring, photographs were taken of the oscilloscope trace to provide a permanent record. For irradiation experiments, the ECG was photographed prior to and immediately after exposure, during any major decline in aortic blood pressure, at the conclusion of the post-irradiation monitoring session, after any drug injections, and during any unusual ECG events. The number of photographs taken during each phase of observation listed above, varied from 1 to several. The time of every photograph with respect to irradiation or drug injection was always recorded.

Ear and Rectal Temperature

The ear temperature was measured with a copper-constantan thermocouple wire (Thermoelectric Canada Ltd) soldered to a 0.1 mm thick, 1.1 mm diameter copper-beryllium disc. The thermal emf was measured and translated into a temperature reading by a digital thermocouple thermometer (AD2036, Analog Devices, USA), and the analog output of this instrument was conveyed to a general purpose amplifier (Beckman, or Gould), and written out by a chart recorder (Beckman, or Gould) as a single, continuous line. The amplifier was calibrated periodically by making gain and baseline adjustments while the ear thermocouple was placed in two water baths whose temperatures differed, but resided between 22 and 40 degrees C. The amplifier and chart recorder were usually set for the recording of temperatures between these limits. The amplifier output, as indicated by the line traced on the paper chart, was tuned according to the digital display on the thermocouple th[illegible]meter. The thermocouple thermometer display provided precision to 0.05 degees C, and the thermometer was accurate to 1.5 degrees C. The two bath temperatures were checked using an alcohol or mercury glass thermometer, which provided accuracy to at least 0.1 degrees C, and could be read to a precision of at least 0.05 degrees C. However, the water bath method was only performed prior to a few experiments, to periodically provide a thorough check of accuracy, while for the majority of calibrations the water baths and glass thermometer were omitted. For the latter, simplified, approach, the accuracy of the amplifier output was checked by comparing the chart recorder line tracing to the digital thermometer display while the thermocouple was held in air, and also while the thermocouple was pressed between two fingers or taped to one of the rabbit's ears. After calibration, the thermocouple disc was taped to the inside surface of the rabbit's left ear pinna, covered with rolled tissue paper, and the ear pinna was taped into a roughly cylindrical configuration. Temperature recording then proceeded. The ear temperature was usually read from the chart recording to a precision of 0.1 degree C (it was sometimes read to 0.01 degree), and the accuracy of the recording system was about 1.5 degrees C. The recording range was generally set for 24-40 degrees C.

Rectal temperature was measured using two different approaches. The first technique employed a copper-constantan thermocouple wire (Thermoelectric Canada Ltd), and was used in 12 rabbits. The thermocouple was sheathed in 1.5 mm OD polyethylene tubing (Clay Adams) which was sealed at the tip with silicone rubber. The thermal emf was translated into, and recorded as degrees C using similar equipment and methods as for the ear temperature. The main difference was that the digital thermocouple thermometer (Model 199, Omega Engineering, Inc.) was more accurate (1 degree C) than that used for ear temperature measurement. Calibration of the system was performed periodically, using the same water bath - glass thermometer approach as for ear temperature recording. The rectal temperature was read from the chart recordings to a precision of 0.01 degree C, and the measurement system provided accuracy to about 1 degree C. The system was set for temperature ranges of 36-44 or 37-41 degrees C. After calibration, the thermocouple sheath was covered with water or petroleum jelly and inserted rectally to a depth of about 10 cm.

The second method of rectal temperature measurement employed a thermistor probe (Yellow Springs #402, Series 400 Temperature Probe, Yellow Springs Instruments, Yellow Springs, Ohio) connected to a temperature amplifier (Gould model 13-4615-47). This approach was used with the great majority of rabbits for which rectal temperature measurement was attempted. The amplifier conveyed the temperature information to the chart recorder pen drive. Initial calibration of the amplifier was effected by replacing the thermistor probe with a resistor. The latter component simulated the electrical resistance which would be generated by the thermistor probe at normal body temperature for the rabbit. A reference chart of resistances was interpolated from specifications provide by Yellow Springs Instruments, to a resolution of 0.1 degrees C The values were generated by executing a SPLINE curve fitting program on an Amdahl 580 computer at the University of Alberta. This program was prepared by Mr L. Coulson of the RRC. After initial calibration, the thermistor probe was connected to the temperature amplifier, and the entire system was calibrated using water baths and a glass thermometer, according to the approach described earlier for the ear temperature. The thermistor probe system provided accuracy to a tolerance of plus or minus 0.1 degrees C, and the temperature was interpolated from the chart recording to a precision of 0.01 degrees C. The system was set to record 2 degree ranges which were usually adjusted to lie between 36 and 40 degrees C. After calibration was completed, the probe was wetted and inserted anally to a distance of about 10 cm.

Computer Directed Physiological Measurement and Recording

For 22 rabbits cannulated in the dorsal aorta, physiological parameters were monitored and recorded under the direction of a computer. The dorsal aorta was cannulated and ECG electrodes were attached according to the methods described earlier in Materials and Methods D. As indicated in Figure 7, the cannula was connected to an external blood pressure transducer (Gould-Statham P23 ID), and the pressure signal conveyed to a strain gauge (Wheatstone bridge) amplifier (Beckman). The blood pressure was then traced onto chart paper by a pen recorder (Beckman R411). The pressure signal was also sent to a digitizer (DAC/ADC converter, Model 3150, Xincom). The digitized blood pressure information was then fed to a minicomputer (980A, Texas Instruments) which, according to program instructions written in both *assembler* and FORTRAN, determined the systolic and diastolic blood pressures and the heart rate. Time signals were supplied to the computer from a signal generator (TM 515 power supply mainframe and TG 501 time mark generator, Tektronix). Alligator clips soldered to shielded turntable tone arm cables were clamped to the rabbit's ECG electrodes, and the voltages were transmitted to a differential AC amplifier (9454, Brookdeal), and then digitized (1170 signal averager, Nicolet). The digitized ECG data were then sent to the computer.

All the parameters mentioned above were sampled for one minute prior to irradiation, and for a 15 minute period commencing at any time after exposure. The data were transferred to a hard disk memory (Disk Drive Model 31F4, Diablo Systems 30, Texas Instruments). The data could at any later time be fed back into the computer and placed on nine track magnetic tape for storage (979 9 Track Magnetic Tape Drive, Texas Instruments). All the recorded data for any experiment could be read from the tape and run through the computer to be printed on paper (X-Y Plotter, Zeta Research) as a series of frames. This was done for 18 of the computer monitored rabbits. Each frame contained data which had been recorded 3 seconds before the data represented in the next frame. The frames included an analog ECG waveform tracing, and also numerical information giving the time relative to exposure, systolic and diastolic pressure, and heart rate.

The blood pressure waveform, and the ear and rectal temperatures were sent directly from the relevant measurement instruments to chart recorder, and were retained in hardcopy analog form on the strip chart. The computer derived heart rate was transmitted to the digitizer and converted to an analog signal. The analog heart rate information went to the chart recorder and was written out as a continuous, single line trace. In order to determine the heart rate the computer tabulated consecutive systolic pressure peaks, and then calculated how many peaks occurred over a given time period. The result was then expressed as the number of heartbeats per minute. For 6 rabbits the computer derived heart rate was compared to another heart rate tracing obtained simultaneously from an ECG/heart rate amplifier (Beckman).

Calibration of the computer to receive blood pressure signals was performed prior to and at the conclusion of each recording session, and was accomplished in concert with calibration of the external transducer - strain gauge amplifier system. The transducer system was arranged and pressurized as described earlier in Materials and Methods. Three successively higher pressures were supplied to the computer, and were identified by typing the numerical values into the computer terminal. The computer, using a program for first order linear least squares curve fitting, calculated the best fit regression line for the three pressures. The computer then interpolated voltage values from the regression line at positions corresponding to the voltage value points generated by the 3 pressures. Subtraction was then performed to determine if, and by how much the measured and calculated values differed. The difference between the pair of numbers showing the widest separation was then divided by the maximum pressure and multiplied by 100 to yield per cent error.

The computer, input/output devices, and connectors and wiring were generously supplied by the RRC. Computer programming and technical expertise were also provided by the staff of the RRC.

Measurement and Recording of Vagal Nerve Transmission

Nerve Cuff Design and Surgical Implantation

Vagal activity was measured by placing a silastic cuff containing three wire electrodes around the nerve. The nerve cuff was developed by Professor R. B. Stein of the Physiology Department at the University of Alberta, and manufactured by Ms. Susan Buller, a member of Dr. Stein's laboratory. The nerve cuff comprised a roughly 1.6 cm long cylinder of medical grade silastic with an internal diameter of about 1.0 mm, and three 75 micrometer thick platinum-iridium (Pt-Ir) wires (Plate 5). The wires were imbedded in the interior cuff surface, and woven to form 3 "C" shaped electrodes. The entire inside surface of each electrode presented a ridge of bare metal protruding slightly into the cuff lumen. A single strand of Pt-Ir wire from each electrode travelled back through the cuff, and the 3 wires were wound about a dacron thread and coated with silastic to form a cable. The cable terminated in a miniature female pin connector (DP-4S-1, Microtech). Additional silastic was evenly applied to the cuff exterior, and the longitudinal band of silastic not interrupted by the electrodes, ie., formed by the open section of the "C" shaped electrodes, was cut through from end to end to allow nerve placement.

Rabbits were starved overnight, anesthetized with halothane gas, and a roll of cloth bandage placed underneath the animal behind the head to maintain the neck in an extended position. The ventral half of the neck was shaved with fine bladed electric clippers approximately from the point of jaw insertion to about 1 or 2 cm above the clavicles. An approximately 3 to 4 cm longitudinal skin incision was made to the left of the neck midline. Overlying fat and fascia were cut, and the left vagus was freed from surrounding connective tissue. Contact with the nerve, particularly with metal instruments, was minimized. Handling of the vagus was effected using smooth glass probes with rounded tips, and which were either straight or curved into hooks. The entire surgical site was frequently irrigated with 0.9% saline, and the nerve cuff lumen was filled with saline from a syringe fitted with a small diameter needle. With the cuff full of saline, the nerve was pushed through the cuff slit into the lumen with a glass probe. The cuff was oriented so that the cable passed caudally out of the surgical opening. The syringe was used to flush saline in and around the cuff, and 3 or 4 strands of 3-0 silk were spaced and tied around the outside of the cuff. The slit was examined to ensure that it was completely closed, and 3-0 silk was looped through one of the cuff ties and a bundle of adjacent muscle and tied. The layers of fascia, the fat, and the skin were each closed separately using interrupted stitches of 3-0 silk. The cuff cable passed out behind the most caudal suture in each stitched tissue layer. The external portion of the cable was coiled, covered with gauze and taped to the chest. In 5 rabbits which had the cuff installed, the dorsal aorta was cannulated with a fluid filled catheter according to the technique described in Materials and Methods D. After surgery was complete, the animals were allowed to recover for several hours in a heated enclosure.

Nerve Transmission Monitoring Apparatus

As shown in Figure 8, the cuff cable pin connector was plugged into a shielded lead which ran to a low noise amplifier (QT 5B, Leaf Electronics Ltd, Edmonton, Alberta) which sent the nerve signal to a differential AC amplifier (9454, Brookdeal). The low noise amplified time domain information was then transmitted to a frequency spectrum analyzer (ARA 412, Communications Company Inc., San Diego, CA), and the resultant frequency versus amplitude profile, ie., power spectrum, was displayed on an oscilloscope (7633, Tektronix).

Calibration of the power spectrum instruments was performed prior to and at the conclusion of each recording session. The calibration unit of a Tektronix 7633 storage oscilloscope was connected to the spectrum analyzer, and the resultant profile conveyed back to the oscilloscope for display on the CRT. The calibration signal comprised a 0.4V peak to peak, 1 kHz square wave with a 3 kHz component. The oscilloscope amplifiers were then adjusted so that the lefthand edge of the 3 kHz bar fell precisely on the tenth horizontal graticle line (see Plate 6). The horizontal lowermost portion of the entire trace was aligned exactly on the 0%, or the lowermost, horizontal graticle line. The horizontal bars or "steps" of the oscilloscope trace represent discrete frequency bands. The ARA 412 is able to measure power amplitude levels for frequencies ranging from 40 Hz to about 16 kHz. The frequency range displayed on the scope for this study was 40 Hz to approximately 5 kHz. The frequency of any band on the CRT display was determined by counting from left to right the number of bands up to and including that of interest, and referring to the manufacturer's frequency versus band-number table. The vertical scale of the ARA 412 output display on the CRT represents volts RMS to the base ten logarithm.

To calibrate the vertical scale of the oscilloscope display of the ARA 412 output, a low frequency signal generator (Model 202C, Hewlett Packard) was connected to the ARA 412. First, the ARA 412 was calibrated with the oscilloscope generated 0.4V calibration signal. Voltage settings on the signal generator were steadily decreased, and were measured using an on line voltmeter (3020, Beckman Instruments). The voltage drop at about 1648 Hz was was measured for every 0.20 cm from the 6 cm horizontal graticle line down the screen to the 0%

level. Also, the measurement error ranges in volts were determined by setting the display bar just below and just above each 0.20 cm line mark on the CRT graticle.

For each of the 13 irradiated rabbits in which power spectrum analysis was conducted, the voltage level displayed by each frequency band between 40 Hz and 5 kHz for all the oscilloscope photographs was determined by first digitizing bar heights in mm using a digitizing table (Calcomp 9000) which fed the information directly into a computer file. The mm values were converted to the corresponding voltages by executing with University of Alberta Amdahl 580 computer, a regression equation calculated from the calibration data of voltage versus trace level.

Verification of the Detection of Vagal Nerve Signals

A 263 day old rabbit was starved overnight, anesthetized with halothane, and the right cervical vagus exposed by Dr R. B. Stein of the Physiology Department at the University of Alberta. Silver (Ag) hook type electrodes were placed in contact with the nerve, and the time domain spectrum photographed from an oscilloscope (7633, Tektronix). A silastic cuff containing 3 Pt-Ir electrodes was then placed around the vagus and the time domain tracing photographed from the oscilloscope. The nerve was crushed distally and the resultant time domain tracing was photographed.

In another type of test, left vagal activity in 8 rabbits was measured with frequency amplitude domain analysis (power spectrum) while the animals were either sedated or killed with sodium pentobarbital. In the first experiment, a silastic nerve cuff was implanted around the left vagus of the animal, and frequency domain analysis was performed. The cuff connector was plugged into a low noise amplifier (QT-5B, Leaf Electronics) which fed the signal into a differential AC amplifier (9454, Brookdeal), which in turn conveyed the amplified measurements into a spectrum analyzer (4441 Miniubiquitous FFT Computing Spectrum Analyzer, Nicolet Instrument Corp.). The latter instrument included a CRT screen which displayed the frequency versus amplitude domain profile. The rabbit was maintained overnight and the power spectrum again obtained using the same equipment, and 0.4 ml sodium pentobarbital (Nembutal; 50 mg/ml Abbott, Montreal, Canada) was injected in the marginal ear vein. The power spectrum oscilloscope display was photographed prior to and immediately after injection. The animal was killed the same day by injection with concentrated sodium pentobarbital (Euthanyl).

A companion experiment to the type described above was performed with seven rabbits, and involved administering a lethal dose of Nembutal or Euthanyl instead of a sedating dose. (Euthanyl contains 240 mg/ml sodium pentobarbital.) All seven rabbits were dead within seconds of injection. A silastic nerve cuff was implanted around the left vagus and connected to a low noise amplifier (QT-5B, Leaf Electronics Ltd) which fed the signal to a differential AC amplifier (9454, Brookdeal). The amplified nerve voltages then were fed into a spectrum analyzer (ARA 412, Communications Company Inc, San Diego, CA), and the resultant power spectrum was displayed on an oscilloscope (7633, Tektronix). Five rabbits were lethally injected with pentobarbital in the marginal ear vein, and oscilloscope power spectrum display photographed prior to and up to 9 minutes after death. In another test, 2 animals were total body irradiated with 1200 cGy at dose rates of 908.8 and 819.2 cGy/minute, in the Gammacell 220. One rabbit was killed after about 90 minutes and the other after 122 minutes post-exposure with Euthanyl given in the marginal ear vein. The oscilloscope display was photographed prior to irradiation, at regular intervals after exposure, and prior to and at various times following injection. In one rabbit the power spectrum was recorded up to 84.5 minutes post-mortem, and for the other the profile was photographed no later than 8.7 minutes after death.

E. Nonphysiological Measurements

Hematocrit Determinations

Thirteen male rabbits ranging in age from 51 to 87 days were used for this portion of the study. Four of the animals were starved overnight, anesthetized with halothane gas, and the dorsal aorta cannulated with a fluid filled catheter according to the method described in Materials and Methods D. After a 4 hour post-operative recovery period the aortic blood pressure was recorded. Nine of the rabbits were neither starved nor subjected to any surgery. Three of the cannulated animals and 6 of the nonoperated rabbits were subjected to TBI with 1200 cGy at dose rates between 1152 and 1155 cGy/minute, in an AEC Gammacell 220. One cannulated rabbit and 3 nonoperated rabbits were sham irradiated; each of these animals was placed in the Gammacell sample chamber for roughly 1 minute without being lowered into the irradiating position.

Using a syringe containing 0.5 ml of heparinized 0.9% saline (150 U sodium heparin/ml, from porcine intestinal mucosa), 2.5 ml blood samples were drawn from the dorsal aorta in cannulated rabbits, and from the central ear artery in nonoperated animals. For cannulated, irradiated rabbits, blood samples were drawn prior to and immediately after irradiation, and during any substantial post-irradiation decline in aortic blood pressure. With the one cannulated animal which was sham irradiated, blood was taken prior to and immediately after sham, and well after the time interval during which hypotension would be expected to occur in an irradiated rabbit. In nonoperated rabbits, blood samples were drawn prior to actual or sham irradiation. A second blood sample was drawn between 57 and 101 minutes after irradiation (or sham). Most of these post-irradiation (or post-sham) samples were taken at about 60 minutes; some were drawn late due to difficulty with the ear artery method, and blood had to be obtained by cardiac puncture. Such large samples (2.5 ml) were drawn because most of the blood was to be used for the determination of plasma prostaglandin levels for irradiated rabbits.

Microhematocrit capillary tubes (3 USP units ammonium heparin/tube, Capilets, Dade, Miami, Fla.) were immediately dipped into the freshly obtained blood samples and approximately two thirds filled. The tubes were closed at the lower end with capillary tube sealant (Seal-Ease, Clay Adams) and centrifuged for about 15 minutes at approximately 441g. Hematocrits were measured as the percentage of packed red cells of total blood volume using a micro-hematocrit reader (Phillips-Drucker). The hematocrit values were all multiplied by 1.2 to compensate for the presence of one volume of heparinized saline for each five volumes of blood.

Lung Histology of Irradiated Rabbits

Ten rabbits were neither subjected to anesthesia nor to surgery. Five of the animals, aged 84 to 294 days, were weighed, and killed by intravenous injection with 1 to 2 ml of a concentrated solution of sodium pentobarbital (240 mg of pentobarbital per ml; Euthanyl, MTC Pharmaceuticals). The lungs, including the trachea and bronchi, were removed immediately, and inflated slowly with 10% buffered formalin delivered by syringe. The open end of the trachea was clamped and the the lungs were immersed in 10% buffered formalin. A second group of five rabbits, aged 53 or 56 days, was subjected to TBI with 1200 cGy at a dose rate of 691.2 cGy/minute in an AEC Gammacell 220. The time period between irradiation of one animal and the next was 20 minutes, and after exposure the rabbits were returned to their cages, and observed for outward manifestations of radiation induced shock. A record was made of each rabbit's condition throughout the post-exposure monitoring period. As soon as rabbits became prostrated and exhibited a lack of responsiveness to external stimuli, they were killed with pentobarbital and their lungs removed and fixed in the same manner as for non-irradiated rabbits. Those animals which did not manifest these signs were killed after about 100 minutes (one animal was killed after between 100-131 minutes) post-exposure, and their lungs removed and fixed. The appearance of the lungs in both irradiated and non-irradiated animals was noted. All the lungs remained immersed in 10% buffered formalin for about one week, after which sample sections were cut from the hilar, middle, and apical regions of each lung.

Histological preparation was performed by Mr R. Mandryk, histologist for the Department of Zoology at the University of Alberta. The fixed lung sections were washed under running tap water for about 1 hour, and then embedded in paraffin. A rotary microtome (Model AO820, American Optical) was used to slice 7 micron sections from each block. Each section was secured on a glass slide, dewaxed with xylene, stained with Masson trichrome, and protected with a glass coverslip. For each lung section 6 to 10 slides were prepared. The slides were examined under low power with a compound light microscope. The proportion of constricted arterioles in each preparation, as evinced by a greatly reduced arteriolar lumen and a thickened muscular layer, was recorded.

F. Protocols of Experiments Performed

Establishing the Cardiovascular Response to Total Body Irradiation

There were 131 rabbits used in this portion of the study, in which cardiovascular collapse was induced by TBI, and the salient physiological characteristics and the frequency of the occurrence of this response were determined.

The arterial blood pressure was monitored either directly with a catheter in the dorsal aorta, or by a noninvasive tail cuff method which measured systolic pressure only. Results were obtained for cannulated rabbits ranging in age from 45 to 935 days, and for cuff monitored animals aged 44 to 93 days. Electrocardiographic, heart rate, and ear and rectal temperature measurements were incorporated at various stages during the project, and adopted as standard practice for cannulated rabbits. The electrocardiogram and ear temperature were also recorded for cuff monitored animals.

None of the rabbits in the aortic cannula group were subjected to any chemical treatments or surgical procedures, other than halothane anesthesia, aortic catheterization~~,~~ and the attachment of ECG electrodes. The nonoperated group received no anesthetic other than topical lidocaine for ECG electrode attachment, and underwent no surgery. Cannulated rabbits were whole body irradiated on the day of surgery in either an AEC Gammacell 220 or a Siemens 6 MeV X-ray machine or an AEC Co^{60} therapy unit. With the Gammacell, doses ranged from between 628.8 and 5088 cGy, at dose rates of 1696 to 892.8 cGy/minute. The whole body dose delivered by the Siemens 6 MeV linear accelerator was 1200 cGy at dose rates of 200 or 91.2 cGy/min. Sixty eight tail cuff monitored rabbits were total body irradiated in the Gammacell 220, with doses ranging from between 4,587 and 978 cGy, at dose rates from 1304 to 1194 cGy/minute. Six cuff monitored rabbits were irradiated with the Siemens X-ray unit at 200 cGy/minute for total body exposures of 1200 cGy or 1600 cGy. Physiological recording was conducted before and for up to two hours after irradiation, and at the conclusion of monitoring most of the animals were killed by an overdose of sodium pentobarbital administered by either intravenous, intra-arterial, intracardiac, or intraperitoneal injection. Forty seven cannulated rabbits were maintained following exposure, observed daily, and their survival times recorded. (A detailed description of this is provided in the following subsection of Materials and Methods.) The 628.8, 2520 and 5088 cGy tests, and 14 of the 1260 to 1200 cGy tests (which produced usable results), were performed by Ms. D. H. Geibelhaus. All these rabbits were cannulated by Ms. E. Dimitrov of the Department of Zoology, at the University of Alberta.

Testing the Influence of General Anesthesia and Aortic Catheterization on Post-Irradiation Survival

The effects of halothane anesthesia and implantation of a saline filled catheter in the dorsal aorta on post-irradiation survival were examined for 61 rabbits ranging in age from 43 to 373 days. Prior to all tests involving anesthesia rabbits were starved, with water, overnight. All treated and non-treated rabbits were maintained on regular food and water supplies and observed daily. The date and time of any death was recorded. For irradiation tests, the animals were exposed 4 hours after the cessation of halothane administration. The experiments performed are described below:

(1) Five 40-100 day rabbits were maintained under halothane anesthesia for 45 minutes, and 4 hours after the cessation of anesthetic delivery were total body exposed to 1102.8 to 1093.2 cGy at 1470.4 to 1457.6 cGy/minute in the Gammacell.

(2) Four 40-100 day rabbits were fed overnight and received no anesthetic. These animals were total body exposed to 1200 to 1092 cGy at 1473.6 to 1456 cGy/minute or at 200 cGy/minute in the Gammacell.

(3) Six 40-100 day rabbits were anesthetized with halothane and the left femoral artery was exposed and tied off. The skin incision was sutured and the animals allowed to recover. Five rabbits were whole body exposed to 1102.8 cGy at 1470.4 cGy/minute, and one animal whole body exposed to 2191.2 cGy at 1460.8 cGy/minute, in ~~the~~ Gammacell.

(4) Four rabbits were anesthetized with halothane, the dorsal aorta cannulated with a catheter containing heparinized saline. These animals were not irradiated, and were allowed to survive unless the hindquarters were paralyzed or the operated leg showed signs of extensive tissue damage. If complications arose, the animals were killed with

Euthanyl.

(5) Sixty five rabbits ranging in age from 43 to 373 days were anesthetized with halothane, the dorsal aorta cannulated with a saline filled polyethylene PE-50 catheter, and a 4 hour recovery period allowed. The animals were subjected to TBI with between 1071.6 to 5088 cGy at dose rates between 1681.6 and 200 cGy/minute, in the Gammacell. The aortic blood pressure in these animals was recorded for up to two hours, and then they were returned to their quarters.

Inferior Vena Cava and Right Ventricular Tests

After catheterizations were complete and the rabbits had recovered from anesthesia, they were taken to the RRC for physiological monitoring. The animals were then subjected to TBI. Four of the IVC catheterized animals were whole body exposed in the Gammacell 220 to between 1181.4 and 1143.3 cGy at dose rates of 756.8 to 738.4 cGy/minute. Twelve rabbits were successfully catheterized in the right ventricle, and 11 of these were irradiated in the Gammacell with doses ranging from 1252.3 to 1192.7 cGy, at dose rates from 710.4 to 697.6 cGy/minute. After TBI the rabbits were returned to the RRC, and physiological monitoring was continued for up to two hours. The animals were killed with pentobarbital at the conclusion of the recording session.

Selective Irradiation/Shielding

Irradiation/Shielding of the Heart Region

The rabbits used in this portion of the study ranged in age from 47 to 69 days, and 15 animals received selective irradiation to the heart region, and 10 were subjected to shielding of the heart area during TBI. The rabbits were starved overnight, anesthetized with halothane, ECG electrodes attached, and a catheter filled with heparinized saline implanted in the dorsal aorta; all according to methods previously described. After a 4 hour recovery period the rabbits were taken to the RRC, and the aortic blood pressure, ECG, ear temperature and for some animals, rectal temperature and heart rate, were recorded. The rabbits were then transported to the Cross Cancer Institute, and subjected to either selective irradiation of the heart region, or total body irradiation with the heart shielded, according to the procedures described in Materials and Methods C. The heart region exposure was 1200 or 2000 cGy at 200 cGy/min with a Siemens 6 MeV X-ray machine, or 1200 cGy at dose rates of 67.2 or 66.6 cGy/minute with an AEC Co^{60} therapy machine (Theratron). With heart shielding, the rest of the body was exposed to 1200 cGy at 200 cGy/min using the 6 MeV unit. The animals were taken back to the RRC and physiological recording was resumed. Recording continued either for about 130 minutes post-exposure, or, with rabbits displaying an abrupt, major drop in aortic pressure, for about 94 minutes. At the conclusion of recording the animals were killed by injection with pentobarbital.

Irradiation of the Head

Three rabbits aged 59, 60, and 74 days were starved overnight, anesthetized with halothane, ECG electrodes attached, and a catheter filled with heparinized 0.9% saline implanted in the dorsal aorta; all according to the methods explained earlier. After a recovery period of at least 4 hours the rabbits were taken to the RRC and the aortic blood pressure, heart rate, ECG, and ear and rectal temperatures were recorded. The rabbits were transported to the CCI and the head exposed to 1200 cGy at 130 or 129 cGy/min with an AEC Theratron 780. The irradiations were performed according to the techniques described in Materials and Methods C. The animals were returned to the RRC, and physiological recording was resumed for between 146.6 to 184.8 minutes. The rabbits were killed with pentobarbital.

Monitoring Vagal Activity and Irradiating Rabbits Fitted with the Nerve Cuff

The nerve cuff was implanted in 43 rabbits, and power spectrum analysis was attempted for 29 of these. Four of the time domain group and 14 of the power spectrum group were

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whole-body exposed to between 1214.2 and 1186 cGy at dose rates ranging between 1011 and 814.4 cGy/min, in an AEC Gammacell 220. Vagal activity was monitored prior to and for up to two hours following irradiation, and oscilloscope photographs of the power spectrum were taken at regular intervals. Recording was usually discontinued about 23 minutes (range: 11-39.5, n=8) after an abrupt fall in blood pressure; when this event did occur it always took place before two hours had elapsed. The majority of rabbits with vagal nerve cuff implants were not cannulated in the dorsal aorta, so that the abrupt fall in blood pressure had to be identified by physical signs observed with over 100 irradiation tests conducted early in the project using rabbits cannulated in the dorsal aorta. The landmark physical signs were sudden prostration, urination, dyspnea, miosis, struggling, and a loss in abdominal muscle tone. Aortic blood pressure was recorded in five power spectrum monitored rabbits, and the ECG was measured in four time domain monitored animals. However, the aortic pressure and ECG were not measured together in the same animals. The ear temperature was recorded for one rabbit in the time domain group. For seven power spectrum rabbits and one time domain rabbit, the signal envelope, i.e., the positive voltages continuously generated by the vagus, was amplified and recorded as a line tracing with the Gould 2400S chart recorder. The positive voltage envelope was traced for 63 Hz and for 1,000 Hz, with each rabbit. Five of the power spectrum rabbits and the time domain rabbit were subjected to TBI.

Unilateral and Bilateral Vagotomy

Surgical Transection of One or Both Cervical Vagi

Rabbits were starved overnight, anesthetized with halothane, ECG electrodes attached, and a catheter filled with heparinized 0.9% saline implanted in the dorsal aorta; all according to the methods described earlier. At some point during anesthesia, usually just preceding aortic cannulation, the ventral aspect of the neck, from the point of jaw insertion to a centimeter or two above the clavicles, was shaved with fine bladed electric clippers. The skin was then swabbed with 70% ethanol, and then for single vagotomy, left or right, a longitudinal skin incision roughly 2 cm in length was made to the side of the midline, towards the vagus to be transected. The layers of fat and connective tissue were cut and pushed aside, and an approximately one centimeter length of vagus was exposed. The nerve was tied with 3-0 silk near each exposed end, and a small section of the vagus cut out. An alternative method was to stretch the nerve over the open prongs of a hemostat, and cut out a short section. The vagus was transected caudad to the separation of the anterior laryngeal vagal branch (N. laryngeus cranialis), and also the separation of the cardiac depressor nerve (N. depressor cardiac). The site was washed with 0.9% saline, any remaining silk removed, and the layers of fat, connective tissue, and skin were each closed with interrupted stitches of 3-0 silk.

Two approaches were used for bilateral vagotomy. The first involved applying the technique described above for unilateral vagotomy to each nerve. The second method was to make a 2 cm lengthwise skin incision in the neck midline and expose one nerve. The nerve was then ligated or stretched over the open prongs of a hemostat, and a short section cut away. The same process of exposure and cutting was used for the second vagus. In two doubly vagotomized animals, the cardiac depressor nerves, which lie adjacent to the vagi in the neck, were also transected.

Monitoring and Irradiation of Vagotomized Rabbits

After surgery the animals were allowed to recover for about 4 hours, although doubly vagotomized rabbits were not given food or water. The rabbits were taken to the RRC and the aortic blood pressure, ECG, ear temperature, and in some cases heart rate, were recorded. The 21 singly vagotomized rabbits were whole body exposed to between 1199.7 to 1179.4 cGy at dose rates of 1152 to 1001.6 cGy/minute in the Gammacell 220. Twelve bilaterally vagotomized rabbits, and two doubly vagotomized animals with both the cardiac depressor nerves cut, were whole body exposed to between 1206.6 and 1166.3 cGy at dose rates of 1136 to 1113.6 cGy/minute in the Gammacell. Twelve doubly vagotomized animals were taken to the CCI and the heart region selectively exposed to 1200 cGy at dose rates between 67.2 or 66.6 cGy/minute delivered by an AEC Theratron 80. Twelve bilaterally vagotomized rabbits were taken to the CCI and the heart region shielded during whole body exposure to 1200 cGy at dose rates of 91.2 to 88.9 cGy/minute, delivered by an AEC Theratron unit. All irradiations were conducted according to the methods described in Materials and Methods C. CCI rabbits were transported back to the RRC following exposure. Physiological recording was resumed for 134 minutes (range: 59-234 minutes, n=40), and rabbits experiencing an abrupt, major drop in blood pressure were monitored for 125 minutes (range: 74-127 minutes, n=13) after exposure. The animals were all killed with pentobarbital.

Interruption of Vagal Transmission by Cooling

Cooling Apparatus

Attempts to reversibly interrupt vagal activity were performed using a metal tipped freon driven cooling device implanted immediately adjacent to the nerve. The entire cooling apparatus was designed, manufactured, and tested by Mr R. J. Gardner and Mr L. D. Coulson of the

RRC at the University of Alberta. The implanted portion of the vagal cooling assembly was constructed of an 8F umbilical vessel catheter (OD 2.7 mm, Argyle), fitted with a thin walled-steel cylinder, the free end of which was soft soldered shut (Plate 7, Figure 9). The cylinder was equipped with two thin wire eyelets, and the cylinder and eyelets were evenly coated with a thin, smooth layer of silicone rubber (Self Levelling RTV, Dow Corning). Stainless steel tubing (0.065" OD, 0.009" wall) was soft soldered to the inside surface of the cylinder and ran back within the lumen of the catheter. Also, contained inside the catheter and soldered to the inside surface of the cylinder, were two thermocouple wires; one was copper, the other constantan. The open end of the steel tubing was fitted to a 1/8" OD stainless steel tube. The catheter was also fitted to a 1/8" stainless steel tube. The stainless steel tubes were sandwiched between two steel plates which formed the connector base. The thermocouple wires were attached to a two pronged plug which also was contained within the connector base. A heating plug was also fitted into the base, and a wire from this plug was attached to the 0.065" OD stainless steel tubing. The unit described in the foregoing was implanted with the cylinder adjacent to the vagus, with the catheter exiting the neck incision caudally, and with the catheter and connector base taped to the chest.

The cooling device tubing connectors were affixed to the receiving ports of two valves (SS-4BMJ Micrometer Needle Valve, Nupro, USA) which communicated with a refrigeration apparatus. The valves were fixed to two 1/4" copper tubes which were about 1 M long; one of these ran to a refrigeration motor (1/3 hp, Tecumseh), and the other to a heat radiator. Liquid freon was driven by the completely sealed, oil lubricated pump into one of the copper tubes. Freon was permitted to flow by opening the two needle valves, and the liquid moved into the 0.065" steel tubing of the cooling probe. The liquid freon left the steel tubing, entered the cylinder volume, and expanded. Expansion was associated with heat uptake, and transformation to the gas phase. The cylinder temperature was measured by a digital thermocouple thermometer (AD2036, Analog Devices). The cylinder thermocouple wire connector was plugged into the thermometer. A mixture of liquid and gaseous freon travelled back within the catheter and past the return valve. The extent of cylinder cooling was dependent on the amount of current passed through the steel conduit tube. Current was supplied to the heating plug by a variac (Super Electric Co.) connected to a step down transformer (Type 1129X, Hammond, 0-4 V ac), which in turn fed a diode. The heating plug was connected to this diode. Current running through the 0.065" steel tubing caused warming of the freon and thereby less cylinder cooling. Eventually, the freon moved through the second copper line and into the radiator. Heat loss resulted in a return to the liquid phase, and the mixture of liquid and gaseous freon travelled back to the refrigerator pump. Water was removed with drying filters, and the freon was frequently recirculated between experiments to rid it of any oil.

Implantation of Vagal Cooling Probe

Seventeen rabbits were starved overnight and anesthetized with halothane. In 7 animals a non-functional version of the vagal cooler was implanted, and 10 rabbits received the operational model. Using fine bladed electric clippers, fur was shaved from the ventral half of the neck roughly from the point of jaw insertion, to one or two centimeters above the clavicles. An approximately one to two centimeter lengthwise incision was made in the skin of the neck to the right of the midline. About one centimeter of the right vagus was exposed and was either tied at each exposed end, or was stretched over the open prongs of a hemostat. A short section of the nerve was then cut away. Then a roughly 2 centimeter longitudinal skin incision was made to the left of the midline. The layers of connective tissue and fat were cut and pushed aside. However, a sheet of fascia overlying the nerve was left intact. The cooling unit was aligned so that the umbilical vessel catheter was directed caudally out of the surgical site, so that the silastic coated steel cylinder was resting against the vagus with the fascia sheet between the cylinder and the nerve.

Surgical silk, 3-0 (Ethicon), was passed through the cylinder eyelets, into adjacent muscle, and tied. Silk strands were also looped around the umbilical catheter, into surrounding muscle bundles, and tied. The layers of fat, fascia, and skin were closed with interrupted stitches of 3-0 silk. The external portion of the umbilical catheter as well as the connector base were covered with surgical gauze and taped to the chest. In several rabbits, four ECG elelectrodes were also attached. The rabbit was then moved to a heated, post-operative enclosure and allowed to recover for several hours. If during the recovery period the animals breathed in a harsh, gasping fashion and evinced distress upon handling, they were killed immediately by injection with concentrated sodium pentobarbital.

After recovery rabbits were taken to the RRC and placed in a metal restraining box. The cooling unit tubing connectors were fixed to the freon system inlet and outlet ports, and the thermocouple plug fitted into the digital thermometer. The thermocouple thermometer digitally displayed cylinder temperature, and also sent the temperature signal to a general purpose amplifier (Gould Instruments Inc.), which directed a recorder pen drive (Gould 2400s) to trace the temperature as a single continuous line on a paper strip chart. Alligator clips soldered to shielded turntable tone arm cables were clamped to the animal's ECG electrodes. The ECG leads were plugged into an ECG/heart rate amplifier (ECG/Biotach, Gould Instruments Inc.), and the heart rate was traced as a single, continuous line on the chart recording. By monitoring probe temperature and heart rate, the extent of heating of the steel freon conduit could be adjusted to yield the degree of cooling necessary to interrupt vagal transmission. Four rabbits were tested on the day of surgery, and each animal was killed the same day with pentobarbital. The cooling unit was immediately removed from each animal and cleaned with soapy water.

G. Chemical Tests

These are organized into two main groups on the basis of the apparent degree of relevance of each agent to radiation hypotension. The chemical tests that seem to bear most directly to the possible treatment of this syndrome are described in the section entitled "Major Chemical Tests", and those drug experiments which are more exploratory are listed in the section called "Exploratory Chemical Tests". The drugs in the section "Major Chemical Tests" are further grouped according to related mode of action, and/or on the basis of whether or not they were directed at the same physiological question(s).

Major Chemical Tests

The drugs referred to in this section were used to address various possible mechanisms for radiation hypotension, and were also tested as possible therapies. The protocol for each drug experiment is given in the subsections which follow. The logic behind each test is briefly described below, and more detailed rationales are given in Results B. One major group of compounds was used to determine if the autonomic nervous system might be involved in the hypotension. Atropine was administered to block cholinergic parasympathetic transmission, and acetylcholinesterase reactivators were given rectify possible significant radiation induced damage to the enzyme acetylcholinesterase (AChE). Propanolol and phenoxybenzamine, respectively a beta and an alpha blocker, were administered in order to oppose sympathetic outflow. Another group of drugs was given to test the hypothesis that histamine might be important in radiation hypotension. Promethazine and Cimetidine, respectively an H1 and an H2 receptor antihistamine, were tested. In a third type of test, digitalis, a drug used to boost the failing heart in heart attack patients, was tested. A fourth approach involved the administration of a corticosteroid, Dexamethasone, to counteract any possible inflammation which might lead to significant edema, and/or to counteract any event(s) occurring in the central nervous system which might lead to hypotension. Finally, it was thought that radiation might cause a release of endotoxin from the gut, or it might cause a reaction similar to the shock mechanism elicited by endotoxin. Hence, endotoxin was given.

Atropine

Seventy four rabbits aged 53 to 91 days were starved overnight, anesthetized with halothane, had four ECG clips attached, and had a catheter filled with heparinized saline implanted in the dorsal aorta; all according to the methods explained in Materials and Methods D. One rabbit also had a PE-50 cannula containing heparinized saline installed in the IVC, also by the technique described in Materials and Methods D. After a recovery period lasting at least 4 hours, the aortic blood pressure, heart rate, ECG, and ear and rectal temperatures were recorded. The ECG waveform was photographed before and after injection, following irradiation, during any major drop in aortic pressure, and at the conclusion of recording. Fifty eight rabbits were injected before and/or after irradiation, either intravenously or intraperitoneally, with 2 to 20 mg/kg of atropine methyl bromide (AMB)(Sigma). Solutions were prepared by dissolving powdered AMB in purified water. NaCl was admixed to produce a 0.9% saline solution, while the concentration of AMB varied roughly between 1 mg/ml to 6 mg/ml. The rabbits were whole body exposed to between 1202.3 and 1131.4 cGy at dose rates from 992 to 894.4 cGy/minute, in an AEC Gammacell 220. Physiological recording continued for up to two hours, and at the conclusion of monitoring the rabbits were killed with concentrated sodium pentobarbital.

The effects of AMB in combination with other compounds was assessed in 6 rabbits. All 6 animals were cannulated in the dorsal aorta with a fluid filled catheter, were fitted with four ECG electrodes, and the aortic blood pressure, ECG, heart rate, and ear and rectal temperatures were recorded, all according to the methods described previously. The animals were subjected to TBI with between 1198.4 and 1175.5 cGy at from 990.4 to 881.6 cGy/minute, in an AEC Gammacell 220. Four rabbits were injected in the marginal ear vein with between 6 and 7.1 mg/kg of a beta adrenergic blocker (propanolol HCl, Ayerst, Montreal) 4 to 10 minutes prior to irradiation. The propanolol was purchased in ampoules containing 1 ml of a 1 mg/ml solution. A fifth rabbit was injected in the marginal ear vein with AMB 2 mg/kg both prior to and after irradiation, and with 1.5 mg of propanolol both before and after exposure. The final animal was injected with 6 mg/kg of AMB, 1 mg of propanolol, and 0.37 mg of digitalis, all intravenously, and all before irradiation. AMB and inderal were prepared as before, and digitalis was purchased in ampoules

(Lanoxin, Burroughs Wellcome, Inc.) containing 2 ml of a 0.25 mg/ml solution. At the conclusion of post-irradiation recording, which lasted for up to two hours, the rabbits were killed with concentrated sodium pentobarbital.

One rabbit was injected in the marginal ear vein with 1 mg/kg of atropine methyl nitrate (AMN) at 8.2 minutes prior to irradiation. The solution was prepared by freshly dissolving 30 mg of powdered AMN in 30 ml of purified water. The solution strength was thus 1 mg/ml. The rabbit was subjected to TBI with 1200 cGy at 992 cGy/minute in an AEC Gammacell 220. The animal was killed at the end of post-irradiation recording with concentrated sodium pentobarbital.

Fifteen rabbits were injected in the marginal ear vein with 1 mg/kg of atropine sulphate between 6.7 and 18.6 minutes prior to irradiation (in one case an injection was also attempted after exposure). The atropine sulphate (Glaxo Laboratories, Toronto, Ontario) was obtained in vials containing 20 ml of a 0.6 mg/ml solution. In one test injection through the marginal ear vein was unsuccessful, so the atropine was delivered through the aortic cannula. In this procedure the drug bolus was followed by flushing with heparinized saline. The rabbits were subjected to TBI with 1186.7 or 1191.7 cGy at approximately 1,000 cGy/minute in the Gammacell 220. At the conclusion of post-irradiation recording, which lasted up to two hours, the rabbits were killed with sodium pentobarbital.

Acetylcholinesterase Reactivators

Nine rabbits aged 52 to 73 days were starved overnight, were anesthetized with halothane, had three ECG electrodes attached, and had a saline filled catheter implanted in the dorsal aorta; all according to the usual methods. Four rabbits to be injected with Diacetyl monoxime (DAM) were also cannulated in the IVC according to the procedure explained in Materials and Methods D. After a recovery period of about 4 hours, the aortic blood pressure, ECG, and ear temperature were recorded. Rabbits were subjected to TBI in the Gammacell at 1134.4 to 1126.4 cGy/minute to yield whole body exposures of 1200.6 to 1197.7 cGy. In five rabbits DAM, at a dose of 72 mg/kg, was given either in the marginal ear vein or through the inferior vena cava, between 5.5 and 9 minutes post-exposure. One rabbit was injected through the IVC with 46 mg/kg DAM at 19 minutes after exposure. DAM solutions were prepared by dissolving powdered DAM (2,3-Butanedione monoxime, Sigma) in water or saline. At the conclusion of post-irradiation monitoring, which lasted up to two hours, the rabbits were killed with sodium pentobarbital.

The immediate toxic effects of pralidoxime chloride

(PAM) were examined in 3 unanesthetized, nonoperated rabbits aged 55, 56, and 156 days. PAM solutions were prepared freshly by obtaining vials containing 1 gm of dry PAM (Protopam Chloride, Ayerst Laboratories, Montreal, Canada) and injecting 20 ml of purified water into the vials. This yielded a solution strength of 50 mg/ml. One rabbit was injected in the marginal ear vein with 50 mg/kg PAM, a second received 100 mg/kg, and a third was given 200 mg/kg PAM. The rabbits were watched for about one hour post-injection, and observed daily for about 4 days. The animals were supplied usual quantities of food and water.

Four rabbits aged 54 to 102 days (only one over 100 days) were starved overnight, anesthetized with halothane, were fitted with three ECG electrodes, and had a catheter filled with heparinized saline implanted in the dorsal aorta; all according to methods previously described. The animals were whole body irradiated in the Gammacell at dose rates of 1140 and 1116.8 cGy/minute, to yield complete exposures of 1204.6 to 1191.3 cGy. One rabbit was injected intraperitoneally 6 minutes prior to exposure with 200 mg/kg of pralidoxime chloride (PAM). The drug was prepared in solution as described above for the toxicity tests. A second rabbit was injected after irradiation with atropine sulphate and with PAM. Three doses of atropine were injected in the marginal ear vein at 13, 77, and 90.7 minutes after irradiation. The atropine was obtained in vials containing 20 ml of a 0.6 mg/ml solution (Glaxo Laboratories, Montreal, Canada). At 19.1 minutes following exposure, pralidoxime chloride (PAM) 200 mg/kg was injected intraperitoneally. The drug was freshly prepared as described earlier.

One rabbit was injected 13 minutes prior to irradiation with 72 mg/kg of pyridine-2-aldoxime Methiodide (PAM, Sigma)[14] into the dorsal aorta. The drug was followed by a flush of heparinized saline. The second rabbit received 72 mg/kg of PAM 18.2 minutes prior to irradiation through the marginal ear vein. These PAM solutions were freshly prepared by dissolving powdered pyridine-2-aldoxime Methiodide in 0.9% saline. At the conclusion of post-irradiation recording, which lasted between about 100 and 120 minutes, all animals were killed with pentobarbital.

Sympathetic Blocking Agents

Five rabbits 78 to 88 days old were starved overnight, were anesthetized with halothane, had four ECG clips attached, and had a catheter filled with heparinized saline was implanted in the dorsal aorta; all according to the usual procedures. After an approximately 4 hour recovery period the aortic blood pressure, ECG, heart rate, and ear and rectal temperatures were recorded. One animal was injected intraperitoneally with 5.3 mg/kg of propanolol HCl, a beta adrenergic blocker, at 9.1 minutes prior to irradiation. The propanolol (Inderal, Ayerst, Montreal, Canada) was purchased in ampoules containing 1 ml of a 1 mg/ml solution. The animal was subjected to TBI with 1191 cGy at 952.8 cGy/min, in an AEC Gammacell 220. At the conclusion of the 78 minute post-irradiation monitoring period the rabbit was killed with sodium pentobarbital.

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[14] PAM from Ayerst and PAM from Sigma are effectively the same drug.

In four rabbits 1 mg/kg of injection grade phenoxybenzamine HCl (Smith Kline and French, Mississauga, Ontario), an alpha adrenergic blocking agent, was given in the marginal ear vein anywhere between 5 to 10 minutes before irradiation. The phenoxybenzamine solution was prepared by dissolving the powdered compound in sterile, purified water to yield a solution strength of 50 mg/ml. One ml of this stock solution, which was stored at at 0 to 5 degrees C, was mixed with 0.225 gm of NaCl in 24 ml of sterile, purified water. This produced an injection solution with a strength of approximately 2 mg/ml. The rabbits were subjected to TBI with 1200 cGy at dose rates of 948.9 and 948.8 cGy/minute, in the Gammacell. The aortic blood pressure, heart rate, ECG, and ear and rectal temperatures were recorded before, during, and after injection, and for up to 2 hours post-irradiation. The animals were killed with concentrated sodium pentobarbital at the conclusion of physiological monitoring.

Antihistamines

The early effects of injecting an H2 receptor antihistamine were tested for in 2 unanesthetized, nonoperated rabbits. The animals received 350 mg/kg of an H2 receptor antagonist (Cimetidine HCl, Smith Kline and French, Mississauga, Canada) intraperitoneally. The antihistamine solution was prepared by dissolving powdered Cimetidine HCl in 0.9% saline, and adjusting the pH to about 6.0 with HCl and NaOH. The rabbits were watched for several hours after injection, and then observed on a daily basis. They were provided with regular food and water supplies.

Four rabbits aged 56 to 71 days were starved overnight, anesthetized with halothane, had three ECG electrodes attached, and had a saline filled catheter implanted in the dorsal aorta; all according to the methods previously described. The animals were permitted to recover for about four hours, and the aortic blood pressure, ECG, and ear temperature recorded. Cimetidine solutions were freshly prepared according to the procedure described above. One animal was injected intraperitoneally with 200 mg/kg of Cimetidine 19 minutes prior to irradiation. Two other rabbits were injected intraperitoneally with 400 mg/kg: one was injected at 55.4

minutes, and the other at 20.2 minutes before irradiation. A final rabbit was injected intraperitoneally with 800 mg/kg of Cimetidine at 20.1 minutes prior to irradiation. The rabbits were exposed to between 1284.4 and 1261.9 cGy whole body, at dose rates from 1190.4 to 1164.8 cGy/minute, in the Gammacell. At the conclusion of post-irradiation recording, which lasted up to 2 hours, the rabbits were killed with concentrated sodium pentobarbital.

The effects of Cimetidine given in combination with an H1 receptor antihistamine on radiation induced hypotension were evaluated in one rabbit aged 51 days. The animal was starved, anesthetized, fitted with ECG electrodes, and cannulated in the dorsal aorta according to the usual methods. The Cimetidine was injected intraperitoneally at a dose of 200 mg/kg, 70 minutes prior to irradiation, and 75 mg/kg of an H1 receptor antagonist was given intraperitoneally at 62 minutes before exposure. The Cimetidine solution was prepared as before and adjusted to pH 5.6, and the H1 antihistamine (Promethazine HCl, Poulenc, Montreal) in powder form was freshly dissolved in water. The rabbit was subjected to TBI with 1200 cGy of 6 MeV X-rays at 200 cGy/minute from a Siemens linear accelerator. At the conclusion of the 122 minute post-exposure recording period, the animal wasa killed with sodium pentobarbital.

One nonoperated rabbit aged 71 days was injected intraperitoneally with 269 mg/kg of Cimetidine 30 minutes before irradiation. The Cimetidine was prepared as usual and adjusted to pH 5.9. The rabbit was subjected to TBI with 1550.8 cGy at 1260.8 cGy/minute in the Gammacell. The systolic arterial blood pressure was recorded prior to and after irradiation with the tail cuff method described in Materials and Methods D. The ECG and ear temperature were also recorded, and the animal was killed at the conclusion of post-irradiation monitoring with sodium pentobarbital.

Dexamethasone

Seven rabbits aged 46 to 80 days were starved overnight, anesthetized with halothane, had four ECG clips attached, and had a catheter containing heparinized saline implanted in the dorsal aorta; all according to methods described previously. After about 4 hours of post-operative recovery the aortic blood pressure, heart rate, ECG, and ear and rectal temperatures were recorded. Five rabbits were injected in the marginal ear vein with about 2 mg/kg of an adrenocortical steroid (Hexadrol; dexamethasone sodium phosphate, Organon Canada Ltd, Toronto) between 360 and 180 minutes before irradiation. The steroid was obtained in 5 ml vials of a 4 mg/ml solution. Two rabbits received 24 mg/kg of the steroid at 180 minutes prior to exposure. The rabbits were exposed in the Gammacell at 1012 to 993.6 cGy/minute for total body exposures of 1200.9 to 1179.1 cGy. At the conclusion of post-irradiation recording, which lasted up to about two hours, the rabbits were killed with sodium pentobarbital.

Digitalis

Two rabbits aged 57 and 58 days were starved overnight, anesthetetized with halothane, had four ECG clips attached, and had a catheter containing heparinized saline implanted in the dorsal aorta; all according to methods previously described. After a recovery period of about 4 hours the aortic blood pressure, ECG, heart rate, and ear and rectal temperatures were recorded. The animals were injected in the marginal ear vein with 0.25 and 0.5 mg of digitalis (Lanoxin, Burroughs Wellcome Inc.) at 8 and 9.8 minutes pre-irradiation, respectively. The digitalis was obtained in ampoules containing 2 ml of a 0.25 mg/ml solution. The rabbits were subjected to TBI with 1175.5 cGy at a dose rate of 881.6 cGy/minute. Physiological recording was discontinued upon death of the animals, at 83.7 and 67.7 minutes post-exposure.

Endotoxin

The immediate toxicity of bacterial endotoxin was assessed in one unanesthetized, nonoperated rabbit aged 106 days. Crystalline flakes of bacterial endotoxin (E. coli lipopolysaccharide, TCA extract, Serotype No. 026:B6, Sigma) were dissolved in purified water to yield a concentration of 1 mg/ml. The endotoxin, 1 mg/kg, was injected in the marginal ear vein, and the rabbit watched. The animal was killed later on the day of injection.

Two rabbits, both aged 66 days were starved overnight, anesthetized with halothane, were fitted with three ECG clips, and had a catheter filled with heparinized saline implanted in the dorsal aorta; all according to procedures described earlier. In one rabbit the IVC was cannulated using the technique explained in Materials and Methods D. After several hours of recovery the aortic blood pressure, ECG, and ear temperature were recorded. Endotoxin solutions were prepared as described above, and 1 mg/kg was injected in the marginal ear vein or via the IVC. Physiological parameters were recorded before, during, and after injection. At the end of the two hour recording period, the animals were killed with sodium pentobarbital.

Exploratory Chemical Tests

The tests in this section differed considerably from one another in terms of rationale. The general basis for each test is described very briefly below, while more detailed background is given in Appendix II. The toxicity and the degree of radioprotection yielded by the sulphydryl radioprotective compound, S-beta, 2-aminoethylisothiuronium bromide hydrobromide (AET) were examined. AET was also given with acetaldehyde in an attempt to reduce its toxicity. Daunorubicin is an anticancer drug that causes various cardiac changes (Smith, 1969). So, the compound was given to young rabbits to ascertain if it would elicit ECG changes and perhaps hypotension, soon after injection. Various enzyme inhibitors were tested to block possible destruction of the peripheral vasculature by lytic enzymes which might released after TBI. Nitrogen mustards produce effects which mimic those of radiation exposure (Elson, 1963). Thus mechloroethamine (a nitogen mustard) was tested to determine if it would elicit hypotension in the rabbit. Histamine was injected in an attempt to produce the physiological changes triggered by TBI. If such changes were elicited by histamine injection, then some support would be provided for the idea that histamine release is responsible for radiation hypotension.

S-Beta, 2-Aminoethylisothiuronium Bromide Hydrobromide - AET

The toxicity of S-beta, 2-aminoethylisothiuronium bromide hydrobromide (AET: Aldrich Chemical Company Inc., Milwaukee, Wis.; Sigma Chemical company, St. Louis, Mo) was tested in 584 separate injections using Japanese quail Coturnix coturnix japonica. Many of the birds used were injected more than once. AET solutions were freshly prepared by dissolving powdered, anhydrous AET in 0.9% saline, and the majority were neutralized with NaOH to pH 7.0 to 7.6. The pH was measured with a pH meter, and checked with pH paper. All vials of AET were stored in vacuum jars which contained the drying agent phosphorous pentoxide. AET which was not in the form of a fine powder and which included lumps was generally not selected for use. During injection sessions, the flasks containing the AET solution were usually kept in ice. The birds were injected peritoneally with AET doses ranging from 75 to 896 mg/kg. The quail were provided with regular food and water supplies, and observed daily. The date and time of any death was recorded. The percentage of deaths out of the injected population was calculated for various time periods after injection. These values were plotted against dose using an APL graphing program run on an Amdahl 470 computer at the University of Alberta.

The toxicity of AET was then assessed with 29 rabbits in 56 separate injections. Many rabbits were injected repeatedly in separate tests. AET solutions were freshly prepared by dissolving anhydrous AET in either buffered or unbuffered saline, and in some cases 1N NaOH (4-5 ml 1N NaOH for 5 gm AET in 10 ml saline) was admixed. AET doses ranged from 30 mg/kg to 800 mg/kg, and were administered either intraperitoneally, intramuscularly, or intravenously. Two rabbits were cannulated in the dorsal aorta according to the technique in Materials and Methods D, and about 4 hours later were injected intravenously with 56.9 mg/kg or 108.7 mg/kg of AET. The rabbit injected with 108.7 mg/kg was subjected to TBI with 1111.2 cGy at 1481.6 cGy/minute in the Gammacell. The animals were watched following injection/irradiation, and then were observed on a daily basis. They were provided with regular food and water supplies, and the discovery date and time of any death was recorded. A third group of AET toxicity tests was performed on 8 rabbits. Each rabbit was treated only once, and two animals were injected with 400 mg/kg, two with 300 mg/kg, and four received 200 mg/kg. The animals were watched after injection, and observed daily. One rabbit in the 200 mg/kg group was autopsied on the day of death. Dr. D. Mackay, Director of Biosciences Animal Services performed the autopsy, and samples were taken of lung, liver, small intestine, kidney, and sternal bone with marrow. The samples were stored in Bouin's fixative, washed with 70% ethanol and 0.1% ammonium hydroxide, and imbedded in wax. Then, 7 micrometer sections were cut, . mounted on glass slides, and stained with Harris's stain.

The effects of AET pretreatment on post-irradiation survival were examined in 8 rabbits. Six animals were administered 112 mg/kg, one received roughly 56 mg/kg, and one rabbit was injected with a quantity of saline equivalent to the 112 mg/kg AET volume. All doses were injected in the marginal ear vein, and at 20 minutes post-injection all 8 animals were total body irradiated in the Gammacell at a dose rate of 1481.6 cGy/minute. Seven of the rabbits were exposed to 1111.2 cGy, and one to 987.7 cGy. The rabbits were watched for several hours after irradiation, and then observed on a daily basis. They were provided with regular supplies of food and water, and the discovery date and time of death was recorded.

The effect(s) of AET pretreatment on radiation induced hypotension was tested in 5 rabbits. Four were aged between 56 and 63 days, and one animal was 110 days old. The animals were starved overnight, anesthetized with halothane, had ECG electrodes attached, and had a catheter containing heparinized 0.9% saline implanted in the dorsal aorta; all according to the methods described earlier. The animals were allowed to recover for several hours in a heated enclosure. The AET solution was freshly prepared by dissolving 1 or 1.3 gm of anhydrous AET powder in 14 or 15 ml, respectively, of 0.9% saline. The pH was adjusted to between 7.0 and 7.3 with NaOH. The solution was then kept in ice. Twenty minutes prior to irradiation, 200 mg/kg of AET (2 to 4 ml) was injected intraperitoneally. The aortic blood pressure and ECG were recorded before and after injection. In 4 tests, the rabbits were taken to the CCI and subjected to TBI with a Siemens 6 MeV X-ray machine at 200 cGy/minute for a complete exposure of 1200 cGy. The rabbits were taken back to the RRC and physiological recording was resumed, and continued for up to two hours post-exposure. Each rabbit was killed with concentrated sodium pentobarbital at the conclusion of recording.

A series of experiments involving AET injection and irradiation of rabbits cannulated in the dorsal aorta, were performed by D. H. Giebelhaus. The data from these experiments are included. The rabbits were cannulated under halothane anesthesia by Ms. E. Dimitrov. AET solutions were freshly prepared by dissolving powdered AET (Sigma) in sterile saline (pH=7.4). Fourteen rabbits between 53 and 84 days old received the AET either intramuscularly or intraperitoneally. Nine rabbits were injected 20 minutes prior to irradiation, and 8 of these received 35 mg/kg AET, and one animal received 30 mg/kg. Three rabbits were injected between 24.4 and 27.7

minutes before TBI, with one animal receiving 35 mg/kg, a second 25 mg/kg, and a third was injected with 15 mg/kg. One animal was injected with 25 mg/kg at 10 minutes before irradiation, and one rabbit received 70 mg/kg at 40 minutes before, and also at 20 minutes after exposure. The animals were subjected to TBI with between 1728 and 1600 cGy at 1296 to 1200 cGy/minute, in the Gammacell 220. Aortic blood pressure was monitored for 2 to 3 hours post-exposure, and the rabbits were maintained until death. However, those animals which exhibited signs of extensive tissue destruction in the operated leg (left leg) such as blackened toenails, cold temperature, lack of sensation and limpness, were killed with pentobarbital. The chart recordings from these experiments were reevaluated and the data stored according to the methods described in section H of Materials and Methods.

The effects of AET pretreatment on radiation induced hypotension were tested in 9 tail cuff monitored rabbits, aged 59 to 77 days. AET solutions were freshly prepared by dissolving powdered anhydrous AET in water or saline. Doses of 200 or 300 mg/kg were given 20 or 30 minutes prior to irradiation. The animals were subjected to TBI with between 1556.6 and 1548.8 cGy at 1265.6 to 1259.2 cGy/minute, in the Gammacell. The systolic aortic blood pressure, the ECG, and the ear temperature were recorded prior to and after injection, and after irradiation. Eight of the rabbits were killed with pentobarbital at the conclusion of physiological monitoring, while one was maintained until death.

Acetaldehyde

The toxicity of acetaldehyde was evaluated in Japanese quail (Coturnix coturnix japonica) using 676 separate injections (many birds were injected more than once). The solution was prepared by mixing 5.6 ml of acetaldehyde (Aldrich) in 50.4 ml of 0.9% saline. The birds were injected intraperitoneally with doses ranging from 11.6 mM/kg to 25.06 mM/kg. The quail were watched for several hours after injection, and then observed daily. They were provided with regular supplies of food and water, and the discovery date and time of any deaths were recorded. Some of these quail were reinjected, and many had also received AET in previous experiments. The toxicity of acetaldehyde was then evaluated for fourteen rabbits. There were twenty-four separate injections; some rabbits were tested more than once. The doses of acetaldehyde were 132 and 154 mg/kg, and were given intraperitoneally. The rabbits were watched after injection and observed daily. They were fed regular rations, and the discovery date and time of any death was recorded.

Acetaldehyde and AET Injected Together

Acetaldehyde and AET were administered together in 100 individual tests using Japanese quail. Acetaldehyde and AET were prepared as usual, neutralized with NaOH, mixed together, and neutralized again with NaOH. The concentration of AET and acetaldehyde were equimolar, and injected at 190 mg/kg and 300 mg/kg. In 19 tests the AET concentration was 300 mg/kg and the acetaldehyde concentration was doubled. The birds were monitored after injection as described above.

Daunorubicin

Two rabbits aged 59 and 60 days were starved overnight, anesthetized with halothane, three ECG clips attached, and a catheter containing heparinized saline implanted in the dorsal aorta. In one animal the IVC was also cannulated with a catheter filled with heparinized saline. Powdered daunorubicin was dissolved in purified water, and injected either through the marginal ear vein or the IVC at doses of 37.3 or 10 mg/kg. The aortic blood pressure, ECG, and ear temperature were recorded before and after injection. The animals were monitored 136.5 and 277.9 minutes following injection, and then were killed with pentobarbital.

Enzyme Inhibitors

One rabbit aged 65 days was starved overnight, anesthetized with halothane, fitted with three ECG clips, and a catheter containing heparinized saline implanted in the dorsal aorta. After about 4 hours of post-operative recovery, the aortic blood pressure, ECG, and ear temperature were recorded. The rabbit was injected in the marginal ear vein with 800 mg/kg of epsilon-amino-N-caproic acid (EACA) 30 minutes prior to irradiation. The EACA solution was prepared by dissolving powdered EACA (ICN, K&K Laboratories Inc, Plainview, NY) in 2 ml of 0.9% saline. The rabbit was irradiated in the Gammacell at 1163.2 cGy/minute for a total body exposure of 1194.2 cGy. Physiological recording continued for 78 minutes post-exposure, and then the animal was killed with sodium pentobarbital.

Six white leghorn chicks 3 days old were obtained from Biosciences Animal Services at the University of Alberta. Soyabean trypsin inhibitor (SBTI) solution was prepared by dissolving 100 mg of powdered crystalline SBTI (Worthington Biochemical Corporation, Freehold, New Jersey) in 10 ml of purified water. Three chicks were injected intraperitoneally with 75 mg/kg of SBTI at thirty minutes prior to irradiation, and 3 chicks received an equivalent volume of 0.9% saline thirty minutes before exposure. The birds were subjected to TBI in the Gammacell at 1168 cGy/minute for a complete exposure of 1,000 cGy. The chicks were observed for roughly 5 hours after irradiation, and those still surviving by that time were killed with pentobarbital.

One rabbit aged 48 days was starved overnight, anesthetized with halothane, fitted with three ECG clips, and a catheter filled with heparinized saline implanted in the dorsal aorta. After a recovery period which lasted about 4 hours, the aortic blood pressure, ECG, and ear temperature were recorded. The animal was injected thirty minutes prior to irradiation through the aortic cannula with 75 mg/kg of SBTI. The drug bolus was followed by a flush of heparinized saline. The SBTI solution was freshly prepared by dissolving 100 mg of powdered SBTI in 10.5 ml of deionized water. The rabbit was subjected to TBI with 1,000 cGy at 1168 cGy/minute, in the Gammacell. ECG photographs were taken only up to seven minutes post-exposure due to technical problems with the ECG. Physiological recording continued for 71 minutes post-exposure, and then the animal was killed with sodium pentobarbital.

One unanesthetized, nonoperated rabbit aged 98 days was injected in the marginal ear vein with 3 ml (60,000 Kallikrein Inactivating Units or K.I.U.) of a polypeptide protease inhibitor and observed. The inhibitor was obtained in ampoules (Trayslol, Bayer AG, Leverkusen, West Germany) containing 10 ml of solution possessing 10,000 K.I.U./ml. A second rabbit aged 47 days was starved overnight, anesthetized with halothane, fitted with three ECG clips, and a catheter containing heparinized saline implanted in the dorsal aorta. The animal was allowed to recover and maintained overnight with food and water. On the day following surgery, the aortic blood pressure, ECG, and ear temperature were recorded. The rabbit was injected in the marginal ear vein with 2 ml (20,000 K.I.U.) of polypeptide protease inhibitor at 10.6 minutes prior to irradiation. Whole body irradiation was performed using the Gammacell at a dose rate of 1177.6 cGy/minute to yield a total exposure of 1216.8 cGy. At the conclusion of post-irradiation recording, which lasted 86 minutes, the rabbit was killed with sodium pentobarbital.

Nitrogen Mustard

The toxic effects of mechloroethamine (nitrogen mustard, HN2) were examined in eight rabbits aged 60 to 97 days. Vials containing 10 mg of HN2 (Boots) in crystalline form were obtained, and the compound reconstituted with 10 ml of 0.9% saline. Alternatively, HN2 was obtained as anhydrous powder (Sigma) and dissolved in 0.9% saline. The drug was injected in the marginal ear vein and doses ranged from 0.2 to 50 mg/kg. The animals were watched for 3 hours following injection, and two rabbits were maintained on regular rations for two days. Three other animals were allowed to live for one day after injection. Surviving rabbits from these two groups were killed with sodium pentobarbital. Three rabbits were killed several hours after injection.

Histamine

Two unanesthetized, nonoperated rabbits aged 104 and 138 days were used to test the immediate effects of histamine given systemically. The solution was prepared by dissolving one gm of histamine in 10 ml of purified water. Then 0.83 ml of the resulting 100 mg/ml solution was diluted to 2 ml with purified water. One rabbit was injected in the marginal ear vein with 0.3 ml of the final histamine solution, which yielded a dose of 7.8 mg/kg. A second animal received 0.1 ml for a dose of 1.8 mg/kg. The animals were observed during the injections.

H. Methods of Data Tabulation and Storage

Blood Pressure Records

Direct Arterial Records

Chart recordings from aortic and right ventricular/aortic monitored rabbits were read for systolic and diastolic aortic pressures using a magnifying lamp (Luxo Lamp, Montreal, Canada) and a transparent sheet marked with pressure gradations matched with the chart scale. For most of the recordings, systolic and diastolic pressures were scored for the following times: pre-irradiation, as soon as possible after irradiation, thirty minutes after irradiation, sixty minutes after irradiation, the point of lowest mean pressure, the point at which mean pressure began to edge down, at the onset of any abrupt major fall in mean pressure, at the end of any abrupt pressure fall, the lowest point of any abrupt pressure decline, the end of the recording, and as soon as a stable pressure had been attained following drug injection. The highest sustained rate of post-irradiation blood pressure decline over at least about 30 seconds was calculated in mm Hg/sec. Any transient, upward or downward spiking excursion in pressure was generally not accepted as representing the true pressure during any phase of the recording. However, in a considerable number of cases with sudden spiking pressures it was difficult to judge whether the sudden excursion represented true pressure or an insignificant transient. In such a case the best estimate of the average systolic or diastolic pressure over a very short time interval the a given point in the recording was tabulated.

Right Ventricular Records

Right ventricular pressures were also read from chart recordings with the magnifying lamp and scale. Systolic and diastolic values and the exact times were scored for the following landmarks: highest pre-irradiation instantaneous and highest pre-irradiation during a 3 minute interval, immediately after irradiation, highest post-irradiation instantaneous, highest post-irradiation over one minute, the highest post-irradiation over 3 minutes, at the onset of both gradual and abrupt systolic pressure rises, at the time of onset of any abrupt major drop in aortic blood pressure, at 30 minutes post-exposure, and at the end of the recording session. The greatest sustained rate of systolic pressure increase was calculated as slope in terms of mm Hg/second.

In order to produce Figures 35 to 44, the right ventricular and aortic pressures were tabulated for each usable right ventricular/aortic recording produced from irradiated rabbits. The instantaneous systolic and diastolic pressures for both the right ventricle and dorsal aorta were digitized for every 0.5 minute mark on each chart recording. This was accomplished using a digitizing table (Calcomp 9000), which transferred the values directly into a computer file. This file was arranged as a single vertical column of related x and y values (assembled as vertical pairs), and was recorded in the operating language (MTS, Michigan Terminal System) of the main computing system at the University of Alberta. For manipulation of the data, and the plotting of Figures 35 to 44, the contents of the MTS file were copied to the filespace of an IBM PC/AT computer.

Indirect Measurements

For cuff recordings, the magnifying lamp and pressure scale were used to tabulate the following parameters: pre-irradiation systolic pressure (usually an average of several cuff readings), systolic blood pressure 20 minutes post-irradiation or as close as possible to this time, time of any abrupt major drop in blood pressure and the recorded pressure closest to this time, approximate time and pressure of gradual decline, time and pressure (if available) of lowest pressure, and time and pressure (if available) of final pressure.

Electrocardiogram and Heart Rate

For all rabbits which had the ECG waveform monitored, the post-irradiation or post-injection waveforms were evaluated for any changes. The ECG waveform photograph exhibiting the greatest degree of change was assigned a numerical value according to the scheme described below: 0 = no ECG change, 1 = mild S-T segment elevation, 2 = substantial S-T segment elevation which was not to level of QRS spike peak, 3 = S-T segment elevated approximately equal to or above QRS spike peak, and 4 = same as #3 but S-T segment and overall waveform alterations extreme and may include cavity potential.

The heart rate was read directly from the chart recording or calculated from the ECG photographs. The highest pre-irradiation heart rate was scored, as were the heart rate immediately after exposure, following drug injection, and the highest heart rate recorded after irradiation. The time of each was also tabulated.

Ear and Rectal Temperature

The ear temperature was scored from the chart recording in terms of the highest pre-irradiation value, the highest post-irradiation value, and the time of onset of the post-irradiation rise. The rate of the post-irradiation increase was calculated in terms of degrees C per minute.

The rectal temperature was read from the chart recordings in terms of the lowest and highest prior to irradiation or injection, the level just after exposure, and the highest (or lowest if a net drop occurred) after irradiation. The rate of the post-irradiation, or post-injection, rise/drop was calculated in degrees C per minute.

Nerve Cuff Records

For each of the rabbits which had left vagal transmission recorded in the form of a power spectrum, and which were total body irradiated, the voltage values represented on each power spectrum photograph were tabulated. For each rabbit, one or more pre-irradiation photographs and all the post-irradiation photographs were used. For every photograph the height of the voltage trace for each frequency was converted to a millimeter value with a digitizing table (Calcomp 9000). The values were transferred from the digitizer directly into a computer file. This file was organized as a vertical column of related x and y values (arranged as vertical pairs), and was recorded in the operating language (MTS, Michigan Terminal System), of the main computing system at the University of Alberta. The numbers for all the rabbits were then copied into the filespace of an IBM PC/AT computer, and converted to microvolt values using a program written in PASCAL by Mr R. J. Gardner of the RRC. Mr L. D. Coulson of the RRC derived the equation used to transform the mm screen height values to microvolts. The data manipulations were performed on the IBM PC/AT computer programmed in PASCAL by Mr R. J. Gardner of the RRC.

Data Storage

Typically 50 to 70 parameters were tabulated for each rabbit and then manually entered into an APL language file residing within the main computing system at the University of Alberta. The title of a given experiment and data from the rabbits used in that experiment comprised a single file. The rabbit identification numbers were arranged in a vector (ie, linear array of numbers in APL), and all the data vectors in the file were constructed so that the data corresponded with the sequence presented by the identification vector. The APL files were copied to the system operating language (MTS) of the main computing system at the U of A so that data manipulations could be conducted using languages other than APL. The APL files and associated MTS file only contain data from rabbits which had at least the aortic blood pressure monitored; data from some of the toxicity tests is not included.

III. Results

A. Cardiovascular Response to Total Body Irradiation

Accuracy and Reliability of Blood Pressure Measurement Techniques

Both the invasive and noninvasive methods of measuring blood pressure generally produced records with good accuracy and little system drift. The evaluation of various drift levels, and the technical basis for the approaches and materials used to measure blood pressure, are presented in detail in Appendix I.

Results of Sham Irradiation

The 10 rabbits in the sham test exhibited an average mean aortic pressure change of +0.7%, the range being -11 to +20.8% (SE=3.15), with no acute responders. Figure 10 is a qualitative depiction of averaged systolic and diastolic values, before and after sham irradiation, for the 10 rabbits.

Cardiovascular Response of Rabbits Cannulated in the Dorsal Aorta to Total Body Irradiation

TBI of young (40-100 day) rabbits with 628.8 to 5,088 cGy frequently resulted in a delayed, sharp fall in aortic blood pressure which generally took no more than about 3 minutes to fully develop, and often resulted in mean pressure which was less than 40% of the pre-irradiation value. Figure 11 was reproduced from the chart record of a 55 day rabbit total body exposed to 1190.4 cGy at 892.8 cGy/minute in the AEC Gammacell 220, and is representative of the response which, in this laboratory, is termed acute cardiovascular collapse, or "ACC". In this report rabbits which experienced ACC are referred to as "acute responders". The topmost tracing in Figure 11 is the aortic blood pressure, and at 66 minutes after exposure there has occurred a steep and substantial decline. The great majority of rabbits were whole body irradiated with doses ranging from 1020 to 1260 cGy, at dose rates of 91 to 1680 cGy/minute. It is this group which is referred to, unless indicated otherwise, in the ensuing description. In these animals the acute pressure drop had a mean onset time of 64.5 minutes after exposure (range: 39.5-111.5, SE=1.8) [15] in 73 rabbits (Table I), and the mean percentage total pressure decline (lowest pre-irradiation to lowest post-irradiation) was 61.8% (42.4-86.3, SE=1.4), in 42 rabbits. This represents a fall of 56.7 mm Hg. The acute drop alone comprised a decline of about 45 mm Hg (11.9-66.6, SE=1.39, n=69) from the mean pressure immediately preceeding the ACC. A gradual decline in pressure often, 97% of the time, developed prior to ACC, so that the total pressure decline caused by irradiation was greater than that effected by ACC alone. In 63% of rabbits displaying ACC, the pre-ACC gradual decline in pressure exceeded 10 mm Hg. The pressure also declined slightly after ACC in 28/68 rabbits. The minimum mean aortic pressure was 34 mm Hg (range: 14-63.2, SE=1.12, n=72), and was attained at about 69.8 minutes (SE=1.7, n=72) after irradiation. By about 96 minutes (range: 51-194) following exposure, the mean pressure recovered to about 51.6 mm Hg (range: 29.3-88.8, SE=1.77, n=72).

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[15] The onset time for ACC given here is from the beginning of irradiation. The value given (64.5 minutes) should be accepted as approximate only, because with different radiation sources the dose rate, and thus the time taken to deliver the complete dose, differed considerably.

During ACC, the blood pressure waveform became much reduced in amplitude and more rounded in shape, and completely lost the dicrotic component. These changes were also noted with rabbits which did not experience ACC, but which did nevertheless suffer profound gradual declines in blood pressure following irradiation. Plates 8 and 9 are photographs, respectively, of the blood pressure waveform before exposure and during minimum post-exposure pressure for an older rabbit experiencing gradual hypotension. ACC was also recorded in rabbits which were fitted with the catheter transducer (Plates 10 and 11), and the pre- and post-irradiation condition of the aortic pressure, ECG, breathing, and muscle tone were identical to those in animals equipped with a fluid-filled cannula.

In Figure 11 the third tracing from the top, the heart rate in beats per minute (BPM), decreased at the time of ACC. However, in general the heart rate tended to increase appreciably during ACC; in 11 of 15 40 to 200 day old rabbits displaying ACC, and which had the heart rate traced on the chart recording, the heart rate rose during the pressure crisis. In one of the 4 animals which did not exhibit a rise in the heart rate during ACC, the heart rate stayed the same, and in another of these rabbits, the heart rate rose during the onset of ACC, then fell. Two rabbits which had the heart rate traced on the chart recording were not included in the analysis because their recordings were not adequate for clear interpretation. The rise in the heart rate which usually accompanied ACC is well represented in Figure 12, which is a reproduction of the chart record of a 110 day old rabbit which experienced ACC after TBI with 1200 cGy of 6 MeV X-rays at 200 cGy/minute. In this recording the heart rate is third from the top, and rises substantially at the time of the acute blood pressure drop (bottom tracing). In some instances the heart rate started to fall as the acute pressure drop ended. In the two rabbits that exhibited a drop in heart rate during the pressure decline, the blood pressure waveform may have become so distorted during the crisis (as often occurred), that the computer missed counting some waveforms, resulting in erroneously low readings.

In 8 of 10 40-100 day old rabbits exhibiting ACC, the heart rate increased by 50.5 BPM (range: 15-64, SE=8.4) within 2.7 to 32 minutes after exposure, while in 1/10 rabbits, the heart rate stayed the same, and in 1/10 animals a decline of 10 BPM was recorded. The highest post-irradiation heart rate in 19/19 animals was elevated by an average 107.3

BPM over the pre-exposure level (range: 35-153, SE=7.37).[16] The highest post-exposure heart rate was recorded at 77.5 minutes (SE=7.8). For the 10 sham irradiated rabbits the heart rate was elevated by more than 10 BPM after sham irradiation in 7 animals, while in one case the rate dropped by 10 BPM. The average increase was 64.5 BPM (range: 55-100) at 11 minutes after sham. The highest post-sham heart rate was elevated by 10 BPM over the pre-irradiation level in all 10 rabbits. The average increase was 64.5 BPM (range: 55-100), at between 3.6 and 119.5 minutes after sham. The average overall increase in the heart rate for the sham rabbits (64.5 BPM) was found to be different from the average overall increase for TBI rabbits (107.3 BPM) at the 1% level of significance, using the Student's t-test. In 6 rabbits the heart rate was determined using both the computer method and the Beckman ECG/heart rate amplifier. In 3 cases either the computer method or the amplifier did not operate properly. In 2 cases both methods functioned during the pre-irradiation interval only, but during this time produced identical records. However, with one rabbit, identical heart rate tracings were produced both before and after exposure, and even during ACC. Hence, the computer and the amplifier techniques substantiated each other.

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[16] The numbers of animals used for the various heart rate calculations differed because not all the heart rate monitored rabbits had the heart rate traced on the chart recording, and not all the heart rate monitored individuals could be reconnected within about 32 minutes of TBI.

In 65% (13/20) of rabbits experiencing ACC, the ECG T-wave exhibited an appreciable to drastic increase in amplitude. This elevation was occasionally accompanied by the evolution of a cavity potential.[17] Plate 12 is the ECG taken from a cannulated rabbit prior to TBI, while Plate 13 is the ECG taken during ACC, clearly showing elevation of the T-wave. During the onset of ACC rabbits often began to struggle within the restraining box, and frequently exhibited dyspnea, miosis, micturition, cyanosis, a striking loss of abdominal muscle tone, and were unresponsive to external stimuli. In some cases the loss of voluntary muscle control was so marked that the animals were completely prostrated. Immediately after irradiation the rabbits often appeared agitated, but the mean aortic pressure was unchanged at 3 to 7 minutes post-irradiation.

For the rabbits of Figures 11 and 12 the rectal temperature (second from the top in both), increased slightly. An increase of roughly 1/2 to 1 degree C was noted in 3/6 rabbits displaying ACC. In all cases, the temperature rise was very gradual. In some rabbits the rectal temperatrure declined slightly during, or just after ACC. The rectal temperature was offset by plus or minus 1 C, however the same direction of systematic error applied to pre- and post-irradiation measurement for each rabbit, so that recorded trends were real. In Figure 11 the ear temperature is at the bottom, and in Figure 12 it is at the top. Both ear temperature tracings rise sharply at the time of ACC; typically the ear temperature was elevated immediately after irradiation, declined substantially after several minutes, and then rose rapidly during ACC. The ear temperature on average (n=13) declined from 33.8 degrees C at 25.7 minutes after irradiation, to 28.1 degrees C by 41.1 minutes--- in 13/15 rabbits the temperature declined by at least 1 degree C. About 93% (14/16) of the rabbits exhibiting ACC also experienced the ear temperature jump during ACC. This sudden rise commenced at 69.5 minutes (SE=4.9, n=13) following irradiation, and constituted a mean increase of 6.9 degrees C (SE=0.88, n=14), with the average rate of increase being 1.57 degrees C/minute. The decline in temperature which was typical following irradiation, was also recorded in some rabbits prior to exposure. However, the sudden ear temperature jump was not observed before irradiation, and none of the sham irradiated rabbits exhibited the abrupt ear temperature rise.

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[17] On an oscilloscope trace the cavity potential appears as a downward spike. This waveform apparently results from the death of a mass of ventricular muscle.

No difference was detected between hematocrits taken prior to and just after irradiation, and during ACC in cannulated rabbits (Table II). Also, no difference between pre-and post-irradiation hematocrits was measured for non-operated animals. Slight changes in the hematocrit were recorded following irradiation, but in order to determine if these were real and separate from the effect of taking blood out of circulation, many rabbits would have to be tested.

This was not done because experiments such as selective irradiation/shielding took priority. The abdominal viscera of irradiated rabbits showed no gross evidence of hemorrhage, such as petechiae, or bloody or non-bloody swelling.

Approximately 73% of the 40-100 day cannulated rabbits experienced the acute blood pressure drop following TBI. Figure 13 is a qualitative depiction of the general blood pressure tracing obtained with acute responders, and was constructed by averaging systolic and diastolic blood pressures prior to and at various times after 6 MeV X-irradiation, for 75 animals exhibiting ACC. This portrayal provides a qualitative impression of the reproducibility of the acute response in terms of character and timing. The average curve for ACC is in contrast to the representation of the averaged blood pressure information for 25 40-100 day rabbits which showed a relatively gradual pressure decline (dotted curve, Figure 13). This type of pressure decline was in this project termed the "deliberate response". The deliberate response followed TBI in 27% of 40-100 day rabbits, and in 11/13 of rabbits aged 100 to 935 days. The deliberate response resembles the post-irradiation pressure declines usually described for the rabbit by previous investigators (see the Discussion). Figure 14 is a reproduction of a chart tracing of the deliberate blood pressure decline which occurred in a young rabbit (less than 100 days old) after TBI. The pressure fall of 14 40-100 day animals showing the deliberate response was 32.1%, or 32.6 mm Hg, while 100-935 day deliberate responders experienced a total fall of 59.5 mm Hg, or 64.5% (range: 45.3-85.4; refer to Table III, and Figure 21). One of seven rabbits in the 40-100 day deliberate response group which had the ECG monitored, experienced T-wave elevation, while one of two animals in the 100-935 day group exhibited the same alteration. Thirteen of seventeen 40-100 day animals experiencing deliberate hypotension had a sharp rise in ear temperature, which averaged 6.8 degrees C (range: 2.8-12.9). The mean onset time for the ear temperature rise was 72.5 (range: 51-107.7) minutes post-exposure. This parameter was not recorded for 100-935 day old rabbits. The difference between the highest post-irradiation heart rate and the pre-irradiation heart rate for 7/7 40-100 day deliberate responders was 79.6 BPM (range: 10-140, SE=20). The time of the highest recorded heart rate averaged 72.1 minutes (SE=15.5) after irradiation. The difference between the highest post-irradiation heart rate and the pre-irradiation heart rate for 3/3 100-935 day deliberate responders was 74.3 BPM (range: 33-100), at between 38 and 65 minutes. Electrocardiographic T-wave elevation occurred in one of two 100-935 day deliberate responders. The few deliberate responders, young and old, which exhibited T-wave elevation suffered very low post-TBI arterial pressure, and some of these animals also experienced dyspnea, emptying of the bladder, loss of muscle tone, and were unresponsive to external stimuli.

Post-Irradiation Survival of Cannulated and Nonoperated Rabbits

Eight cannulated rabbits died during post-exposure blood pressure monitoring. Seven of these were less than 100 days old, and 4 of these animals experienced ACC. Three of the entire group of 8 were subjected to total body doses in excess of 1,550 cGy.

The long term effects of halothane anesthesia and implantation in the femoral artery or dorsal aorta, of a catheter filled with heparinized saline were examined. These tests were performed to determine if long term survival studies could be coupled with post-irradiation blood pressure experiments. The results of the survival tests are presented in Table IV. The mean survival time of 4 non-operated, non-halothane exposed rabbits which were whole body irradiated with 1092 to 1200 cGy was 8.1 days. Cannulation of the femoral artery diminished survival to 1.57 days after irradiation, in 48 43 to 98 day old rabbits. The reason for this is unknown, although possiblities may include disruption of aortic blood flow by the cannula, or complications arising from flushing with saline which contained heparin. Non-irradiated cannulated animals did not survive past about 4 days post-operation, although work done in this laboratory has revealed that cannulated rabbits can recover full use of the operated leg and survive many months; the operated leg must be adequately exercised. The results of these survival tests however, indicate that long term studies of rabbits cannulated in the dorsal aorta and whole body irradiated with at least 1092 cGy, might not be practicable.

Cardiovascular Response to Total Body Irradiation of 40-100 Day Tail Cuff Monitored Rabbits

ACC in cuff monitored animals was principally identified by a sudden loss of the tail pulse accompanied by rapid elevation of the ECG T-wave and/or ear temperature (Figures 15 and 16). Lesser in importance but still of assistance, was the occurrence either singly or in any combination, of dyspnea, struggling, miosis, micturition, cyanosis, and a marked diminution of abdominal muscle tone. As shown in Table V, the frequency of ACC was over 50% in the 950 cGy to 2,850 cGy dose category.[18] This is about 20% less than the frequency observed using cannulated animals. This may reflect a somewhat lesser tendency for nonoperated rabbits to suffer ACC, or it may at least in part derive from limitations of the cuff technique as a method by which to distinguish acute from deliberate hypotension. Although many cases were clearly deliberate or acute responders, in some tests, ambiguity did exist, and may have caused the frequency of ACC to be underestimated. Also, the pre-irradiation systolic pressures were significantly lower in cuff rabbits than for cannulated rabbits (see Tables I and III). The onset time of ACC differed by 6.6 minutes between cuff and cannula groups. The basis for this difference may in part reside with the fact that often the exact onset time for ACC could not be determined using the cuff technique. Some delay usually occurred because an extensive loss of the pulse signal, appreciable ECG T-wave elevation, and other changes, had to clearly develop, before acute hypotension could be confidently identified. This partial explanation is supported by the finding that the mean onset time for the ear temperature rise did not differ between cuff monitored and cannulated rabbits. The ear temperature jump with cannulated rabbits took place at approximately the same time as the onset of ACC. However, in cuff rabbits the ear temperature rise preceeded the onset of ACC by 4 minutes, suggesting that perhaps the onset time for ACC in these animals was overestimated. The extent of the ear temperature elevation differs between the cuff and cannula groups by a small but statistically significant margin. The final systolic pressure does not differ between cuff and cannulated rabbits. Table III shows that the depth of deliberate hypotension does not differ significantly between cuff monitored and cannulated rabbits: the minimum post-irradiation systolic pressure and the mean percentage total decline in mean aortic pressure are very close for the two groups. This is rather unexpected as the pre-irradiation systolic pressure was higher in cannulated deliberate responders than in tail cuff animals, by the same mean value as was recorded for acute responders. The final systolic pressures were very close between cuff and cannula groups.

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[18] This value should be regarded only as approximate, it could be higher or lower. This is due to the uncertainty in classifying some of the recordings as acute or deliberate.

Although the tail cuff method provided periodic systolic pressure only, and seldom provided readings when the pulse was weak during hypotension, and was not as accurate as the invasive method, the single important objective of this approach was attained: to provide proof that post-irradiation hypotension and specifically ACC occurs in previously normal, non-operated rabbits, and is not an artifact caused by anesthesia and surgery prior to irradiation. Although the incidence of ACC may be somewhat reduced for cuff monitored animals, there is no doubt that this response does occur in considerable numbers of nonoperated rabbits.

Graphical and Numerical Analysis of the Cardiovascular Response to Total Body Irradiation

Statistical analyses of the maximum rate of the post-irradiation blood pressure decline, or slope in mm Hg/second, reveal that in general ACC either happened or it did not happen; relatively few rabbits experienced blood pressure reductions that were intermediate between deliberate and ACC. This point is illustrated by the graphical presentations in this section. The dramatic all-or-none nature of the acute cardiovascular response suggested that relatively few rabbits would likely have to be tested in order to ascertain the effects of experimental treatments; a larger number of deliberate responders would be needed to discriminate between subtle gradations in the extent of gradual hypotension. Due to the foregoing, and for reasons described in the Discussion section of this report, a major objective of this study was to maximize the frequency of acute radiation hypotension to create as effective an experimental assay system as possible. Thus detailed quantitative analyses on the incidence and characteristics of ACC were performed to ascertain which rabbits would be more likely to be acute responders. The analyses presented in this section almost exclusively refer to rabbits which were monitored using the invasive approach. Data obtained from cuff monitored animals are identified as such.

Age Dependence

Figure 17 shows that ACC occurred chiefly in immature rabbits, while older animals tended to exhibit deliberate pressure reductions after whole body irradiation. The graph was constructed by first calculating from post-irradiation recordings, the highest sustained mean rate of decline of arterial pressure. Then for each rabbit the rate, or slope in mm Hg/second, was divided by the animal's age in days. The vertical axis comprises the base ten logarithm of slope divided by age. The horizontal axis represents age in days, and is scaled to the base ten logarithm. Points were plotted according to the slope/day figures and ages of 109 rabbits ranging in age from 45 to 935 days. Rabbits with the steepest slopes are represented in the upper lefthand quadrant of the graph, and the overwhelming majority belongs to the 40-100 day age group. Slope values were divided by age to enhance presentation of the data, but the age effect is equally as statistically significant when slope alone is plotted versus age. The middle line represents the regression, and the outer two lines indicate the points which were included in the regression calculation. The group of rabbits less than 100 days old, residing in the lower left quadrant of the graph were not included in the regression, as these were found to comprise a statistically separate group from the rest of the 45 to 100 day rabbits, at the 1% level of significance (Student's t-test). In any case their inclusion in the regression calculations would not greatly change the slope of the regression line as most of the points for 40 to 100 day rabbits are concentrated in the upper left quadrant of the graph.

Figure 18 provides further evidence, in terms of the rate of the blood pressure fall, for the existence of two discrete subpopulations within the 40-100 day age group. Three acute responders and one deliberate responder were not included in this graph. The left vertical axis represents the culmulative number of rabbits for any point on the solid line, and the right vertical axis indicates to the base ten logarithm the number of rabbits corresponding to each bar. The horizontal axis is the base ten logarithm of slope in mm Hg/second. The solid line was produced by plotting in sequence from shallowest to steepest, the slopes for all the 40 to 100 day control rabbits; any point on the line represents the culmulative number of rabbits from left to right. A single, clean point of inflection is evident in the line at a slope value of 0.20 mm Hg/second, dividing the rabbits into two groups. This proves the bimodal character of the 40-100 day group with respect with the cardiovascular response to TBI. The bars denote the numbers of rabbits in various slope categories, and offer a pictorial representation of the slope distribution.

Rabbits having slopes of greater than 0.20 mm Hg/second and which exhibited a sustained drop in mean aortic pressure of more than about 20 mm Hg, were categorized as acute responders. Any animal which exhibited a maximum slope of less than 0.20 mm Hg/second was classed as a deliberate responder, even though the slope in question may have belonged to a sustained decline which resulted in profoundly low arterial pressure. Twenty five deliberate responders younger than 100 days had average slope of 0.058, while the acute responders less than 100 days which exhibited a group average of 0.615. The difference between these two groups is significant (1% confidence level, Student's t-test). ACC occurred in 73%, 75 out of 103, 40-100 day rabbits, while only 2 of the 13 rabbits older than 100 days exhibited slopes equal to or greater than 0.20. '' The average slope for 40-100 day acutes was 0.615, while the mean slope for 100-935 day deliberate responders was 0.073 (SD=0.065): the difference is statistically significant at the 0.01% level (t-distribution). Figure 19 shows the average blood pressure curve obtained in 11 100-935 day deliberate responders, following TBI with 1200 cGy of 6 MeV X-rays. The age category cutoff was designated as 100 days rather arbitrarily--the two acute responders in the older slope/age group were aged 110 and 131 days, which was well past the age, 99 days, of the nearest younger rabbit showing ACC. The cutoff could in fact lie anywhere between 99 and 142 days without materially affecting the interpretation of the data presented in Figures 17, 18, or subsequent analyses.

Dose Response and Response According to Radiation Source

Table V shows the proportion of acute responders in various dose categories for cannulated and cuff rabbits aged 40-100 days. The results of grouping the cuff and cannula animals are shown for each dose class. Figure 20

''There is a discrepancy in the numbers presented here which is due to the fact that some animals died, and others had incomplete pressure records due to clotting. True slopes could not be calculated for these animals, but most of them showed deliberate pressure declines. Thus the incidence of acute hypotension is 75/103, but slopes were calculated for 100 rabbits in the 40-100 day group.

was constructed using the data from column 1 and column 4 of Table V. The horizontal axis shows dose to the base ten logarithm, horizontal axis is to the base ten logarithm so that each dose category and the vertical axis indicates the frequency, in per cent, of ACC out of the total number of animals tested in each dose range. The proportion of acute responders is 60% in the 6 to 9.5 Gy dose range, but this should be accepted as approximate due to the small number of animals tested. In the 9.5 to 15.5 Gy dose class, 68.8% of the animals exhibited ACC, while 63.3% of the rabbits in the 15.5 to 28.5 Gy group displayed the acute response. In the 28.5 to 50.9 Gy dose range, 46.7% of the rabbits experienced ACC. It is interesting that increasing the dose did not raise the incidence of acute shock. Therefore, 9.5 to 15.5 cGy appears to represent a range of doses which elicit ACC relatively frequently; elevating the exposure above 15.5 Gy would not increase the frequency of ACC.

Table VI indicates the frequency and depth of hypotension for rabbits irradiated with the 6 MeV X-ray machine, the Gammacell 220, and the AEC Theratron (80 and 780). The frequency and the depth of ACC is the same for all three groups. However, the time of onset of ACC is later with rabbits irradiated with the AEC Theratron 80 and 780. The possible reasons for this are described in Discussion A.

Results of Experiments

Figure 21 is referred to extensively in this section. The horizontal axis is in percent. The vertical axis separates various experimental conditions. The solid black bars show the proportion of rabbits experiencing ACC out of the total number of animals tested in each treatment category. The total number of rabbits is indicated at the righthand end of every black bar. The hatched bars depict the average percent difference between the lowest pre-irradiation and the lowest post-irradiation mean aortic pressure for all the rabbits, acute and deliberate responders, in each experimental group. The standard error of the mean is indicated on each hypotension bar. Only results for rabbits cannulated in the dorsal aorta are portrayed, and with the exception of the topmost pair of bars, they represent 40-100 day old rabbits. Figure 21 reveals that TBI of rabbits older than 100 days with 1200 cGy resulted in an average blood pressure decline of 69.1% (SE=3.31), with 15.3% (2/13) of the animals experiencing ACC. TBI of 40-100 day animals with between 1020 and 1260 cGy, resulted in a mean total decline of 55.4% (SE=2.25) and a 73% incidence of ACC.

Selective Irradiation/Shielding

Heart Irradiation/Shielding

When rabbits with intact vagi were irradiated over the heart region--- results 1200 and 2000 cGy exposures were pooled-- a 29.2% (range: 7.9-58.3, SE=3.7) decline in mean aortic blood pressure was recorded, and the incidence of ACC was 1 out of 15, or 6.7%. Figure 22 shows the averaged blood pressure tracings for the 14 deliberate responders and for the one acute responder. The deliberate curve is similar to, but shallower than that for TBI rabbits exhibiting deliberate hypotension, while the acute curve resembles that for TBI acute responders. ECG T-wave elevation was recorded with 2 of the deliberate responders, but not in the acute responder. The ear temperature was not measured in the acute responder, but the ear temperature jump was recorded for some deliberate responders.

The use of fluoroscopy is essential for proper localization of the heart; in one instance, fluoroscopy revealed an approximately 2 cm error with the non-fluoroscopic method. One of the 3 rabbits prepared without fluoroscopy exhibited an acute drop in blood pressure. The other two rabbits displayed a slight increase, 2.6%, in mean aortic pressure after exposure, while the acute responder experienced a 58.2% decline. Although the non-fluoroscopic approach involved error, the results are significant in that they show that irradiation of a relatively small volume in the vicinity of the heart can cause ACC.

Heart shielding during total body exposure to 1200 cGy in rabbits with intact vagi resulted in an average overall pressure decline of 19% (SE=6.3). One of 10 rabbits (10%) suffered ACC (Figure 21). Figure 23 shows the average blood pressure tracing for the 9 deliberate responders and the one acute responder. The appearance and depth of the deliberate hypotension curve is similar to the deliberate curves for TBI and for selective heart irradiation, while the ACC profile resembles those obtained with heart exposure and with TBI. These results are significant in that shielding of the heart region alone markedly diminished the average pressure decline and the frequency of ACC. The heart irradiation/shielding experiments show that the heart region, which includes the heart, roots of the systemic vessels, pulmonary artery and vein, bronchi, part of the lungs, and the thymus, is a key target in terms of radiation hypotension. Animals showing the deliberate response after whole body exposure with the heart shielded did not exhibit any ECG change and did not present any sudden ear temperature rise. ECG T-wave elevations were observed for the acute responder and for one steep deliberate responder. Both these rabbits exhibited the sudden ear temperature rise.

Irradiation of the Head

The results of head irradiation are not shown in Figure 21. Following exposure of the head alone to 1200 cGy, the mean aortic pressure in 3 rabbits declined an average of 6.1% (range: -14.8 to +3.8, SE=5.4). The ECG and ear temperature did not show sudden, significant change. The average decline in mean aortic pressure following head irradiation did not differ much from that for sham irradiated rabbits; one of the head irradiated animals exceeded the maximum pressure decline exhibited in the sham group, by 3.8%. One of the head irradiated rabbits experienced slightly increased pressure after exposure. The head does not seem to be a highly sensitive target.

Unilateral and Bilateral Vagotomy

Unilateral Vagotomy

Following TBI, right cervically vagotomized rabbits experienced an average post-irradiation decline in mean aortic pressure of 44.9% (SE=3.81), which is slightly less than the hypotension seen with non-vagotomized TBI animals (Figure 21). The difference however, is statistically significant. The incidence of ACC was 70% (7/10)--- the same as with no vagotomy. ECG T-wave elevation occurred in 5/7 acute responders, and 1/3 deliberate responders. The ear temperature jump occurred in

5/7 acute responders, and 1/3 deliberate responders. For left cervical vagotomy the fall in mean aortic pressure was 45.1% (SE=4.5), with 64% (7/11) of the rabbits exhibiting ACC. ECG T-wave elevation and the sudden ear temperature rise were observed for 3/7 and 5/6, respectively, acute responders, and 3/3 and 3/4 respectively, deliberate responders.

Bilateral Vagotomy

Double cervical vagotomy prior to whole body exposure to between 1206.6 and 1194.4 cGy, reduced the extent of the radiation induced decline in mean aortic pressure to 24.9% (SE=3.7), with none of the 11 rabbits showing ACC. Figure 24 represents the average pressure tracing for the 11 animals, and reveals that the pulse pressure, or difference between systolic and diastolic pressures, did not diminish following irradiation. This is in contrast to the pulse pressure reduction which occurs in the overwhelming majority of deliberate and acute responders following TBI, heart irradiation, and TBI after single vagotomy. ECG T-wave elevation was not recorded for any of the rabbits, while the sudden ear temperature rise was observed in three animals. In three rabbits a sudden, marked rise in the aortic pressure commenced between 38 and 92 minutes after irradiation. The pulse pressure was expanded, and the overall appearance of the tracing was much like that recorded after endotoxin injection (section III B). The dyspnea which invariably resulted from double vagotomy seemed to be aggravated by irradiation. The fact that serious breathing problems arise from double vagotomy means that the interpretation of all experiments using bilaterally vagotomized rabbits must be tempered. For further discussion of this point the reader should refer to the section on vagal cooling (section III A). The finding that double vagotomy abolished ACC and reduced the depth of hypotension may implicate vagal participation in post-irradiation hypotension.

Double Vagotomy and Heart Irradiation

Exposure of the heart region alone to between 1190 or 1200 cGy in rabbits which had both vagi transected, caused an average decline in mean aortic pressure of 14.8% (SE=2.57), with no acute responders. Figure 25 is the averaged blood pressure tracing for the entire group. ECG T-wave elevation was observed in 2/12 rabbits, and the sudden ear temperature rise was recorded in 5/12 animals. The overall pressure decline in all the animals differed, by a statistically significant margin (0.010% level of significance, t distribution), from rabbits which were subjected to heart irradiation and which had both vagi intact. Two of the rabbits exhibited a sudden, rise in mean aortic pressure and in pulse pressure at about 73.5 minutes after irradiation. One of these animals experienced the sudden rise in ear temperature at the onset of the pressure rise. The pressure tracing resembled that seen following endotoxin injection (section III B). The results indicate that the vagi participate when the heart alone is irradiated, but also demonstrates that when the vagal influence is removed, the heart region still shows some sensitivity to radiation.

Double Vagotomy and Heart Shielding

Shielding of the heart region in doubly vagotomized rabbits reduces aortic hypotension resulting from TBI to 9.9% (SE=4.03), with no acute responders. It should be noted that the range of blood pressure change shown by these rabbits is +7 to -32.2%. Two of the rabbits exhibited hypotension of 32.2 and 27%, while for all the rest of the animals the hypotension did not exceed 15%. No ECG T-wave elevation nor a jump in ear temperature were recorded in any of the 10 rabbits. The hypotension developing in doubly vagotomized rabbits which had the heart region shielded is significantly less than that occurring with the double vagotomy group, but it is not significantly different from the hypotension recorded following TBI with the heart region shielded (Figure 21). Figure 26 represents the average blood pressure tracing for the entire group, and shows that the pulse pressure remains undiminished after irradiation, and that the aortic pressure falls slightly. Two of the rabbits exhibited a sudden rise in the mean aortic pressure and in the pulse pressure, at about 81 minutes after exposure. One of the animals experienced the sudden ear temperature rise during the onset of the pressure increase. The blood pressure tracing resembled that seen following injection with endotoxin (section III B).

Figure 27 shows that for TBI, heart irradiation, and TBI with heart shielding, double vagotomy reduced the extent of hypotension. The vertical axis shows the lowest post irradiation mean aortic pressure as a percentage of the lowest pre-irradiation pressure. The lowest pressure is plotted for the three types of irradiation versus the number of vagi cut. In all cases double vagotomy raised the minimum pressure. The degree of protection provided by single vagotomy, right or left, is less than half that conferred by bilateral vagotomy. Each vagus seems to contribute equally to the hypotension, so that to some extent the effects of vagotomy are additive. The presence of cross innervation may account for the fact that double vagotomy is more protective than the per cent improvements of left and right single vagotomy added together.

Monitoring of Vagal Transmission Using the Nerve Cuff

Verification of the Nerve Signal

Silver (Ag) electrodes in direct contact with the exposed right vagus of a rabbit under halothane anesthesia facilitated measurement of how electrical potential associated with the nerve changed with time; this is referred to as time domain recording. Plate 14 shows modulation in the time domain CRT tracing; the larger amplitude clusters corresponded temporally with the animal's breathing. The Ag electrodes were then replaced by a silastic cuff containing three Pt-Ir electrodes, and as shown by Plate 15, modulation in the electrical activity of the vagus was again evident with the increased amplitude bursts appearing in unison with breathing. Plate 16 shows how the signal modulation disappeared after the vagus was crushed distally. A similar result was later obtained with another rabbit under halothane anesthesia, using a silastic cuff. The Nicolet 444A spectrum analyzer was used initially, and Plate 17 is the time domain tracing obtained from a fully conscious rabbit on the day of cuff implantation. Plates 18 and 19 are voltage amplitude versus frequency profiles for the same rabbit as in Plate 17, taken, respectively, on the same day and on the day following placement of the silastic cuff around the left vagus. The vertical axis indicates voltage and is scaled to base ten logarithms, and the horizontal scale is the frequency in

Hertz, also to the base ten logarithm. A bipartite profile is apparent, with the lefthand bell curve most probably representing electromyographic (EMG) voltages produced by skeletal muscle activity, and the righthand bell curve likely resulted from potentials associated with vagal transmission. Plate 24 is a time domain profile for a second rabbit produced the day of cuff implantation. Both the vertical and horizontal scales are logarithmic in this profile. In this power spectrum the EMG and nerve curves show peak frequencies at 40 Hz and 1600 Hz, respectively. Potentials arising from muscular activity are of much lower frequency than nerve signals, so that in Plate 24 the wide separation between the two curves indicates their respective identities. Further evidence for the identities of the two curves was generated by tests in which the rabbits were injected with various doses of sodium pentobarbital. The rationale behind these experiments began with the idea that sedation would abolish voluntary muscle activity, thereby greatly reducing or even eliminating EMG noise. Consequently if the lefthand curve in the vagus power spectrum was EMG noise, it would drop considerably or perhaps disappear. Vagal activity would be expected to continue, possibly somewhat abated, so that the righthand power spectrum curve should remain relatively unchanged if it was vagal in origin. Plate 20 is the power spectrum for the same rabbit as in Plate 19, and was taken shortly after intravenous injection with sodium pentobarbital. In Plate 20 the lefthand curve has vanished while the righthand curve remains. The sedating dose of pentobarbital greatly reduced voluntary muscle activity and the breathing was much slowed. Therefore the lefthand curve is presumed to represent EMG noise, while the righthand curve represents continuing vagal transmission. This test was repeated in another rabbit using a higher total dose of pentobarbital and achieved very comparable results. In this test however, the power spectrum contained a substantial amount of interference which filled the region between the EMG and nerve peaks (Plate 22). The EMG component was unusually low. Following pentobarbital the interference occupying the middle frequency range vanished, the putative nerve profile remained and was well defined, and the EMG curve persisted at about its pre-injection amplitude (Plate 23). Hence the source of the mid-range interference was likely due to muscle activity. Further testing with this particular rabbit could not continue due to equipment malfunction.

Similar tests with lethal doses of pentobarbital excluded the possibility that residual muscle activity in sedated animals contributed to the righthand curve. Plate 24 shows a left vagal power spectrum derived with the ARA 412 spectrum analyzer on the day of cuff implantation. About one hour after producing Plate 24, the mid-range between the EMG and neural peaks filled. Intravenous injection with concentrated pentobarbital (Euthanyl) instantly killed the animal, and Plate 25, photographed immediately, shows that only the neural component of the power spectrum persisted. Vagal activity slowly diminished until the profile appeared as in Plate 26, taken at 6.5 minutes post-injection. These results were closely reproduced in two non-irradiated and in two whole body irradiated rabbits. The latter were lethally injected with pentobarbital at about 122 minutes following exposure. Attempts to duplicate the test in two additional non-irradiated rabbits failed to reveal a discrete vagal component in the power spectrum. However in one of these experiments the frequency profile before pentobarbital provided little indication that nerve signals were at all being detected, and it appeared that one of the cuff leads was damaged. The profile obtained with the second rabbit prior to injection also seemed rather poor and did not exhibit apparent neural voltages (Plates 27, 28, 29, 30).

The righthand frequency domain curve which remained following pentobarbital was not an artifact produced by noise from the measurement electronics. Plate 31 depicts the frequency domain profile at 1.5 minutes after injection with Euthanyl. The pre-injection power spectrum was almost identical to that in Plate 31, as this particular rabbit was very quiet and calm. Plate 32 is the CRT display with the nerve cuff disconnected from the rest of the measurement system; the tracing clearly does not resemble the spectra shown in the plates which were taken after rabbits were killed.

Cuff implantation may have damaged the left vagus to some extent, as trials in which the vagal cooler was placed against the left vagus with the right vagus transected, revealed that installation of the cooler caused nerve damage. However, in many instances cuff installation did not appear to completely suppress vagal transmission, as a definite nerve power spectrum was observed. Recognizable bipartite frequency domain profiles were obtained up to about two days following cuff installation, but could not be recorded beyond this time. The presence of the cuff may have caused any, none, or all of the following: (1) progressive damage to the vagus from continual pressure and abrasion; (2) persistent pressure on the nerve may have impeded axoplasmic transport and/or the blood supply; and (3) post-operative infection may have caused deterioration of nerve tissue. Table VII summarizes the results obtained with six rabbits for which left vagal activity was monitored for at least one day following cuff installation. In 2 out of 5 tests, good signals were recorded the day of surgery, in 3 of 6 rabbits good signals were evident one day after surgery, while in 2 cases the power spectrum was poor at 4 days after implantation (Plate 21). The majority of rabbits both had the left vagus monitored and were irradiated on the day of surgery.

Nerve Cuff Recording Results for Total Body Irradiated Rabbits

The total surgical time for cuff implantation was relatively long due to the delicacy of the procedure, with operations typically lasting about one hour. Five out of the 14 rabbits which were used for power spectrum vagal nerve monitoring were cannulated in the dorsal aorta; these animals are identified in the ensuing presentation of results. The tail cuff technique was not used during vagal recording because cuff inflation would prompt the rabbit to fidget, thereby generating considerable EMG noise. The ECG, rectal temperature, and ear temperature were not recorded for any of the power spectrum monitored animals. This was to minimize possible electrical interference of the nerve signal created by various measurement devices: the nerve voltages could easily be obscured by other voltage sources as they were very small--- less than one microvolt.

Evidence of ACC was observed in 6 of the 14 power spectrum monitored rabbits; more animals may have experienced the acute response but since very few were cannulated, and none were fitted with ECG electrodes or tail cuff blood pressure monitored, it was not possible to ascertain this.

After the number of microvolts in each frequency band in each power spectrum photograph taken for every rabbit was determined, the data was grouped according to whether or not the rabbits exhibited ACC, and on the basis of the EMG noise content of the power spectra. The six acute responders comprised one group, the two deliberate responders whose power spectra contained relatively little noise formed another, the four deliberate responders from which poor recordings (high noise level, low nerve profile amplitude) were placed in a third group, and the one rabbit, a deliberate responder, which showed a definite decline in the neural peak voltage following TBI was by itself. One of the two low noise deliberate responders showed a decline in the neural peak amplitude from the pre-exposure level, after TBI. However, the recording for this animal was poor. It should be noted that the animals termed deliberate responders are presumed to have experienced deliberate hypotension; there was no way of determining whether they all actually had any hypotension.

The data presented in Figures 28 to 31 were prepared according to the approach described below. First, the microvolt value for each frequency band for power spectra taken at various times after TBI had subtracted from them the corresponding band values of the pre-irradiation power spectrum which possessed the highest nerve peak and which was relatively noise-free. The same pre-irradiation spectrum was used with each of the selected post-irradiation spectra for a given rabbit. The resultant values for each frequency band were then divided by the pre-irradiation values and multiplied by 100 to yield per cent change. The per cent values for each set of power spectra were then averaged. The sets of power spectra were; (1) within 10 minutes of TBI, or as early as possible after TBI; (2) roughly 50 to 60 minutes after TBI (3) near the end of the recording period, or at the time when the nerve profile was at its maximum amplitude.

Eleven of the 13 rabbits (which provided usable records) showed at some point during the post-irradiation monitoring period, a neural peak amplitude in the power spectrum which was greater than the pre-irradiation level. Two of the 13 animals exhibited a lower neural peak amplitude after TBI than prior to exposure. Figure 28 shows the averaged per cent change in the power spectra for the 6 acute responders at 3 different times after irradiation. The vertical axis denotes positive and negative per cent change, and the horizontal axis is the frequency (scaled to the base ten logarithm). Each symbol represents the average per cent change for a particular frequency band. The symbols are joined by lines as a visual aid. It can be seen in Figure 28 that all the frequency bands above 1 kHz showed a positive change in terms of per cent. This means that the voltage in those frequency bands was higher than the pre-irradiation level for the times indicated. The EMG bands (around 80 Hz), were also elevated after irradiation. However, for 9.5 and for 70 minutes after exposure, the neural bands were elevated (particularly for 70 minutes), the EMG bands were also elevated, but the bands residing within the middle frequency region (200 Hz to 800 Hz) either stayed the same or dropped, compared to the pre-irradiation level. This means that the rise in the low frequency EMG voltages did not spill over and elevate the neural bands; the mid region bands which are of lower frequency than the nerve profile would be affected first, instead they declined in amplitude.[30]

Figure 29 is constructed in the same way as Figure 28, and shows the averaged per cent change in the power spectra bands at various times for 2 deliberate responders which exhibited little EMG interference. The neural power spectra were all elevated at each time following irradiation, the low frequency EMG bands were also elevated, but the mid frequency bands declined after TBI. Again, the latter finding indicates that the increase observed for the neural voltages was not produced by EMG spillover. Figure 30 shows the averaged per cent change in the power spectrum frequency bands for 4 deliberate responders whose power spectra contained relatively high levels of noise, or which had low neural profile amplitude. The neural voltages for these animals increased after TBI, but so did the voltages for the middle frequency range. The latter may have been caused by potentials generated by muscular activity. It is possible that voltages spilled over from the upper mid region into the neural bands, thus causing them to rise. However, the separation between the putative EMG frequencies and the presumed neural frequencies was large, so perhaps only relatively weak voltages belonging to higher EMG signal region harmonics would have reached the neural bands. Figure 31 shows the per cent change in the power spectra for the deliberate responder which exhibited a decline in the height of the neural peak following TBI.

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[30] Most, if not all the rabbits exhibited greatly elevated EMG voltage amplitudes when they shifted about in the restraining box. At these times the neural voltages were swamped by EMG voltages spilling into the higher frequency bands. This shows that if the EMG potentials were sufficiently large, the neural frequency bands could be affected.

The range of per cent change in the third (final) power spectrum for Figures 28, 29, and 30, is shown in Appendix IV by, respectively, Figures 54, 55, and 56. The per cent change for the final time is plotted for each rabbit individually. Hence the data of Figures 54 to 56 was averaged for each group of rabbits to produce the final time plots in Figures 28 to 30. The ranges for the final time were portrayed because the increase in the neural peak amplitude over the pre-irradiation level was the greatest at this time. Only one animal is represented in Figure 31, making it unnecessary to show the range.

Five of the 6 rabbits which had the vagal voltage envelope traced for both 63 Hz and 1 kHz exhibited a rise in the 1 kHz trace over the pre-irradiation level. The other rabbit displayed a definite, steady post-irradiation increase in the height of the 1 kHz tracing, but the height of the post-exposure tracing was not as great as the highest pre-irradiation level. In all rabbits the amplitude of the 63 Hz tracing (in the EMG region) was elevated above the pre-irradiation level, but in four cases the 63 Hz tracing showed declines at some point(s) after TBI while at the corresponding time(s) the 1 kHz trace either rose or stayed at the same level. In two animals the 63 Hz and 1 kHz tracings, at least during some periods of the recording, rose and fell together. The finding that the 1 kHz envelope tracings for all six rabbits increased steadily after irradiation indicates that the power spectrum photographs from four of the rabbits[31] chosen to contribute data for Figures 28 to 30 were not transients, in the sense that the elevated neural voltages they indicated were not anomalies after TBI.

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[31] Two of the six rabbits were not included in power spectrum measurement and/or analysis.

Vagal Cooling

Preliminary, non-functional versions of the vagal cooler were installed next to the left vagus, accompanied by transection of the right vagus, in seven rabbits. Dyspnea was taken as evidence that the left vagus had been damaged, and appeared in four of these animals. The fully operational cooler was implanted in ten animals, and seven of these evinced dyspnea. One of these seven, and the three rabbits breathing normally had the left vagus cooled. Only one rabbit displayed a discernable, clean response. This animal was not dyspneic after surgery, and rapid cooling of the left vagus (the right vagus was cut), caused heart rate elevations which averaged 38.3 BPM (range: 15-58) on 10 cooling runs. Figure 32 is a reproduction of the chart recording for this test, and shows that the heart rate rose and fell according to the probe temperature. The heart rate usually began to rise when the cooler temperature was between roughly 0 and -12 C. After the eighth cooling run the heart rate declined more slowly after the cooler was shut off, suggesting that the nerve may have been damaged. Probe temperatures of -10 to -20 C were attained several times, and during one run -35 C was reached, so the vagus may have been frozen. Since the temperature only of the probe was known, and because the cooler tended to drop to low temperatures very rapidly, it was difficult to appropriately regulate cooling. However, the rise in heart rate recorded during cooling runs was likely due to cooling of the nerve, and not a product of any discomfort caused the rabbit by cold cylinder tip. This seems to be the case since a rabbit which had the cooler accidentally placed too far from the vagus to cool the nerve, exhibited no change in heart rate when the cooler was operating.

The successful test represented in Figure 32 was followed by eight failures. In seven of the animals the left vagus was damaged by probe installation, and in one case the cooler was too far from the nerve to effect sufficient cooling.

Pressures in the Inferior Vena Cava and in the Right Ventricle of Rabbits Subjected to TBI

Results of Pressure Measurement for the Inferior Vena Cava

The cannula tip rested roughly between about 2 and 5 cm from the anterior margin of the heart in 2 of the irradiated rabbits. In a third case the catheter was too far from the heart to yield a good pressure recording, while in a fourth animal the IVC transducer zero drifted very high. Only two usable IVC pressure recordings were obtained, and the two rabbits aged 71 and 85 days, belonged to the irradiated group. One animal did not have the arterial pressure monitored either by direct or indirect means, but manifested physical signs of ACC following exposure, and the post-exposure mean IVC pressure rose to double the highest pre-irradiation level (Table VIII). The second rabbit was also cannulated in the dorsal aorta and exhibited a rapid deliberate decline in arterial pressure, and the mean IVC pressure increased by 84% over the highest pre-irradiation mean.

Right Ventricular Pressure Results

Excellent pressure recordings were obtained from the right ventricle provided that blood thrombi did not form at, or within the catheter tip. During right ventricular recording, the aortic pressure was measured with the Millar catheter tip transducer outside the animal, as described in Materials and Methods D. This method yielded significantly lower arterial pressures than those obtained with the Gould P23 ID external transducer, as revealed by comparison of Tables I and III to Table IX. The basis for this difference is not known. Possibly the apparatus which connected the cannula and catheter transducer was not sufficiently pressure tight; it was occasionally observed to slowly leak. Or, perhaps the presence of a catheter within the right jugular and right atrium affected homeostatic mechanisms and/or disrupted cardiac dynamics, resulting in unusually low arterial pressures. Notwithstanding, acute and deliberate post-irradiation hypotension did develop in right ventricular catheterized rabbits, and the average decline in mean aortic pressure was 46.8% (range:36.9-52.6, SE=2, n=8). This did not differ by a statistically significant margin from the 55.4% average decline recorded for whole body exposed, 40-100 day rabbits which were cannulated in the dorsal aorta only. Figure 33 is a reproduction of the chart recording of a rabbit catheterized in the dorsal aorta and in the right ventricle, and total body irradiated in the Gammacell. The aortic blood pressure shows an abrupt fall, and well before this event, the right ventricular pressure rises sharply. Figure 34 is a reproduction from the chart recording of a deliberate responder which was cannulated in the right ventricle and in the dorsal aorta, and which was subjected to TBI in the Gammacell. Four of the 10 right ventricular pressure monitored rabbits, for which usable pre- and post-irradiation pressure recordings were obtained, experienced the acute blood pressure fall. In three of these the right ventricular pressure rose sharply before the acute aortic drop. The right ventricular pressure began to sharply move up 4.3 (SE=1.25) minutes before ACC commenced, and by the onset of the acute drop the right ventricular pressure had attained about roughly 90% of its maximum systolic height. For one acute responder the right ventricular pressure did increase after irradiation, but the recording was poor, and it was not possible to tell when the pressure rise commenced. Two rabbits experienced steep deliberate aortic pressure declines, and four displayed deliberate pressure reductions. Table IX shows the results for right ventricular pressure monitoring of TBI rabbits. One acute responder is not included in the tabulations because the recording was not satisfactory, although it gave indication that the right ventricular pressure rose after TBI. Also, one of the rabbits died after irradiation and the pressures of course dropped to zero, so this animal was not included in the calculation of the total per cent drop in mean aortic pressure; 8 rabbits were used to determine this value. The rise in mean right ventricular pressure averaged for the entire group was 45.3%. This represents a rise from the average pre-irradiation mean pressure of 12.5 mm Hg (SE=0.88, n=9), to 17.4 mm Hg (SE=0.96, n=9) after TBI. A Student's t-test revealed that these pre- and post-irradiation averages are different at the 1% level of significance. Eight of the 10 rabbits exhibited a rise in right ventricular pressure, and 2 animals showed diminutions of 12.5% and 4.4%. Both these animals were deliberate responders. When these 2 rabbits are not included in the calculations, the averaged pre-irradiation mean right ventricular pressure is determined as 11.8 mm Hg (SE=0.95, n=7), which rises to 18.4 mm Hg (SE=0.90, n=7) after TBI. For the entire group of rabbits (n=9), the systolic right ventricular pressure show a substantial rise from the pre-exposure average of 36.8 mm Hg (SE=2.4) to the post-exposure average of 52.6 mm Hg (SE=2.8).

It should be noted that the labored breathing which develops during deep hypotension may be partly responsible for the the increased amplitude of the right ventricular pressure trace after TBI. Recent work in this laboratory with very fast chart recorder paper feed rates, has shown that during the labored breathing associated with hypotension, the right ventricular pressure trace is superimposed on a continous sine pattern which is produced by respiration. Thus when run at low chart speed, the amplitude increase in right ventricular pressure is exaggerated. However, the mean pressure is not markedly affected by this phenomenon, and in most rabbits does exhibit a definite rise after TBI.

Figures 35 to 39 were produced by averaging the numbers obtained from the digitization of both aortic and right ventricular systolic and diastolic pressures. The pressures were digitized for each 0.5 minute mark on each chart recording. All the data manipulations were performed on an IBM PC/AT computer according to a program written in PASCAL by Mr R. J. Gardner of the Radiation Research Center at the University of Alberta. Figure 35 includes averaged right ventricular and aortic pressure values for 3 acute responders. The 3 rabbits represented in Figure 35 all produced good right ventricular pressure records. The computer located the time at which the peak right ventricular pressure occurred after irradiation for each rabbit, calculated the average time between the 3 records, and shifted the right ventricular and aortic data for each rabbit so that the peak right ventricular pressures for all 3 corresponded. The degree of shifting involved was not large, and this was done so that the similarity between the 3 records would be evident; otherwise averaging would round off and diminish various sharp, characteristic features. After shifting, the right ventricular and aortic pressures were then averaged and plotted. A 20 minute pre-irradiation time interval was digitized for each rabbit, these were averaged, and the average values plotted to lie between -20 and 0 minutes. This latter manipulation was done for Figures 35 to 39. Figure 35 shows clearly how for the acute responders, the right ventricular pressure began to rise very sharply before the onset of ACC. Figure 36 is a plot of right ventricular and aortic pressure with time for the one acute responder which produced a poor right ventricular recording. It can be seen that the right ventricular pressure appeared to rise after TBI, but the recording is erratic. Figure 37 was produced for 2 steep deliberate responders. The computer determined the times at which the aortic systolic pressure began to shoulder down, calculated the average for the 2 rabbits, and then shifted the aortic and right ventricular data for both rabbits to correspond to the average time. Note that the right ventricular pressure begins to rise rapidly well before the aortic pressure enters the steep portion of its decline. Figure 38 portrays the averaged pressure data for two deliberate responders, and involved no shifting of the data. Figure 39 comprises the averaged pressure data for the 2 deliberate responders for whom the right ventricular pressure after irradiation was less than the highest pre-exposure level.

The second set of manipulations which was performed was intended to determine if the ratio of mean right ventricular pressure (MRVP) divided by mean aortic pressure (MAP) changed after irradiation. The rationale for this rested with the concept that if blockage to pulmonary flow does occur after irradiation, the right ventricular pressure should rise and the aortic pressure should decline. In such a case the ratio MRVP/MAP would increase. On the other hand if the aortic pressure decline were due to causes other than blockage to pulmonary blood flow, the right ventricular pressure may drop substantially, and the ratio (MRVP/MAP) would not rise. For example, if extensive peripheral vasodilatation caused low arterial pressure and heart failure due to reduced coronary perfusion, it would be expected that the right ventricular pressure would drop not rise. Thus the ratio MRVP/MAP should not rise.

For each group of rabbits represented in Figures 35 to 39, the shifted (or nonshifted) pressure values were used by the computer to calculate mean right ventricular and mean aortic pressures. The mean right ventricular pressures were divided by mean aortic pressures, and the resulting numbers were plotted versus time post irradiation in Figures 40 to 44. The mean aortic pressure was also plotted on each graph. Figure 40 was drawn for the 3 acute responders, Figure 41 for the acute responder which produced a poor right ventricular record, Figure 42 for the 2 steep deliberate responders, Figure 43 for the 2 deliberate responders, and Figure 44 for the 2 deliberate responders which exhibited a decline in right ventricular pressure after irradiation. Note that all the graphs indicate that the ratio MRVP/MAP increased after irradiation, even for the 2 animals which exhibited right ventricular pressure declines. This suggests that pulmonary blood flow may have been impeded.

Results of Lung Histology

Microscopic examination of the lung tissue sections from irradiated and non-irradiated rabbits revealed that the exposed group had a higher incidence of arteriolar constriction, but the results are inconclusive. Arteriolar constriction occurs in non-irradiated lungs, but a key question is whether this is spread through part or all of the lung. Many irradiated and non-irradiated rabbit lungs would have to be tested in order to overcome the limitations of sampling. Also, some of the animals should be killed by a method other than injection with an overdose of pentobarbital to exclude the possibilty that this drug causes constriction of the pulmonary arterioles. However, even after extensive, careful effort, the histological approach would still be of limited value. This point is referred to again in the Discussion.

B. Results of Chemical Tests

Those drug tests which are directly relevant to the findings for selective heart irradiation/shielding and right ventricular measurements, and which were conducted thoroughly, are presented in this section. The data from chemical tests which were more exploratory are given in Appendix II. The results from different types of drugs are often presented in groups, due to related mode of action, or because they are directed at the same physiological question. The rationale for each drug test is briefly described.

Major Chemical Tests

Atropine

Atropine was given to block the cholinergic efferent vagal fibres, and three different versions of this drug were used. The first is atropine sulphate which crosses the bloodbrain barrier and cannot be used in doses greater than 1 mg/kg (Goodman Gilman et al, 1980; Bland). The second is atropine methyl bromide (AMB), and the third is atropine methyl nitrate (AMN). The last two are quaternary forms and were used because they are unable to cross the blood brain barrier, which might allow differentiation between central and peripheral processes, and because they can be administered in much higher doses than atropine sulphate (Goodman Gilman et al, 1980; Bland). With all types and doses of atropine a definite pharmacologic response was recorded immediately after injection. The heart rate tracing became smooth and stable, and rose by 85.4 BPM (range: 44-116) in 8 rabbits which received 2 mg/kg of AMB. The heart rate rose by 110.7 BPM (range: 60-164) in 12 rabbits which were injected with 1 mg/kg of atropine sulphate. The pupils usually dilated, so to minimize ocular discomfort for the rabbit, the restraining box was kept under low light by covering it with heavy cloth. The atropine results were pooled to construct Figure 45. Various doses of atropine are plotted versus the lowest mean aortic pressure (averaged for all rabbits in each drug dose category for both deliberate and acute responders together), and are also plotted against the frequency of ACC. The overall post-irradiation pressure fall was reduced from 55.4% for the non-atropine control group, to 41.4% when 6 mg/Kg atropine was given. The incidence of ACC dropped from 73% with the controls, to about 26% when 6 mg/Kg of atropine was administered. The latter result is consistent with the findings with double vagotomy, and points to the importance of the vagi in deliberate, and particularly in acute hypotension. The post-TBI blood pressure was not raised much with 6 mg/Kg atropine (41.4% of pre-TBI), and was significantly lower than that developing in doubly vagotomized rabbits (roughly 74% of pre-TBI level). Three out of 5 acute responders which were injected with 6 mg/kg atropine exhibited the sudden jump in ear temperature, and all 5 of these rabbits displayed elevation of the ECG T-wave. With 10 mg/kg AMB 1 rabbit died, and the results for hypotension and the frequency of ACC lie well outside the limits suggested by the data up 6 mg/kg atropine.

Acetylcholinesterase Reactivators

Results from double vagotomy and atropine indicated that parasympathetic release of acetylcholine plays an important role in acute radiation hypotension. A drop in the levels of the enzyme acetylcholinesterase (AChE) following irradiation has been reported for humans (Barnard,1948). Exposure of AChE *in vitro* with 2,000 cGy of 45 kvp X-rays was found to cause at least a 30% reduction in the activity of the enzyme (Hasson-Voloch et al, 1973). Irradiating solutions of AChE with a very high dose of Co^{60}, 30,000 cGy, has produced conformational changes (Nayar and Srinivasan, 1975). Despite the fact that the literature is unclear, it was decided to test the effects of an AChE reactivator on radiation hypotension, TBI of the guinea pig with 400 cGy has been reported to cause by 24 hours post-exposure, a rise in the AChE activity in the bone marrow, and a slight decline in the plasma AChE activity (Lundin, 1960). with the idea that radiation might affect the functioning of AChE causing the rabbits system to contain an excess of ACh. None of the five rabbits receiving diacetylmonoxime (DAM) exhibited less than the expected level of hypotension following whole body irradiation, and 4 out of 5 animals experienced ACC. Two acute responders experienced the ear temperature jump, and one exhibited ECG T-wave elevation. The average total drop in mean aortic pressure was 60.7%. The three animals treated with PAM (either pyridine-2-aldoxime methiodide or pralidoxime chloride) showed an average aortic pressure reduction of 58.4% (range:39.1-70.9), with one acute responder. The ECG and ear temperature were not monitored in this animal. A final rabbit, injected with PAM as well as atropine given serially, experienced a deliberate decline in aortic pressure of 60.6%. This animal displayed both ECG T-wave elevation and the ear temperature jump.

Sympathetic Blocking Agents

Propanolol HCL, a beta adrenergic blocker, was tested alone, together with atropine methyl bromide, and with both atropine methyl bromide and digitalis, to address the possibility that efferent beta adrenergic activity contributes to radiation hypotension. In one animal 5.3 mg/Kg propanolol did not block acute hypotension, and at the time of the acute crisis the ECG T-wave rose, but the ear temperature increased very slightly. In four rabbits, 6 to 7.1 mg/Kg of propanolol and 1.5 mg of atropine methyl bromide had no effect on the depth of hypotension, and 2/4 animals experienced ACC. Both acute responders experienced the sudden ear temperature jump, and one acute responder displayed ECG T-wave elevation.

Atropine methyl bromide, 4 mg/kg, and 3.0 mg of propanolol failed to block ACC and did not significantly, if at all mitigate the hypotension. When atropine methyl bromide, 6 mg/Kg, propanolol, 1 mg, and 0.37 mg of digitalis were given together, the animal died at 73 minutes after irradiation. The results suggest that beta adrenergic blockade, alone or together with atropine, does not diminish radiation hypotension. However, when atropine and propanolol were given together, either one or the other drug was adminstered in a relatively low dose to avoid potential circulatory system complications. In one test, both drugs were injected at high dose, but digitalis was also given and the animal died after exposure. It was found that rabbits treated with digitalis alone died after irradiation. Both atropine and propanolol could have been tested together at high doses in several rabbits, although the uniformly negative results obtained with propanolol did not seem to justify this.

Treatment with 1 mg/kg of phenoxybenzamine HCL, an alpha adrenergic blocker, in four animals failed to prevent ACC or to significantly lessen the extent of radiation hypotension. In the acute responder the ear temperature showed an extremely slight rise suddenly during the pressure drop, and in one steep deliberate responder, who might be expected to exhibit the temperature jump, the ear temperature remained stable. In all 4 rabbits the ear temperature remained high throughout the post-irradiation recording period; it did not show the usual post-irradiation decline. The highest average pre-exposure ear temperature was 37.4 degrees C, and at 13.2 minutes after irradiation it was 37.1 degrees C, and by 39.8 minutes it had gradually diminished to 35.9 degrees C. The results suggest that the ear temperature rise which is usually recorded with acute responders is due to alpha adrenergic sympathetic activity, and that blockade of the alpha adrenergic system does not change the characteristics of post-irradiation hypotension.

H1 and H2 Receptor Antihistamines

Histamine has been postulated by several investigators to be the primary factor responsible for post-irradiation hypotension (Painter et al, 1946; Weber and Steggerda, 1949; Doyle et al, 1974; Alter et al, 1983). Consequently, two antihistamines were tested. One rabbit was injected with promethazine HCl, an H1 receptor antihistamine, together with cimetidine HCl, an H2 receptor antihistamine. The rabbit experienced drowsiness after injection, and the mean aortic pressure was rather low, 63.9 mm Hg. The average pre-irradiation mean aortic pressure for 43 non-injected, cannulated rabbits was 93.3 mm Hg (range: 73.2-126.9, SE=1.68, SD=11). The antihistamine treated rabbit therefore had low pressure, which probably was due to the H1 blocker, as cimetidine injected alone in other rabbits did not cause drowsiness or low blood pressure. Promethazine is known to have potent sed~~ating~~, central effects (Goodman Gilman et al, 1980). After irradiation the aortic pressure dropped by 27%, to 46.6 mm Hg. The pressure decline was deliberate in character. Only one animal was tested so no conclusions can prevent be drawn as to whether this combination of antihistamines can ACC, but certainly deliberate hypotension was not prevented. The test results are complicated by the fact that the H1 antihistamine appeared to lower the pre-irradiation blood pressure. Presumably, the drug had significant central nervous system effects, so even if ACC were to be abolished in further experiments, the mechanism of action would be unknown.

Injections of cimetidine, an H2 receptor antihistamine, did not affect blood pressure or cause drowsiness. Cimetidine prevented neither acute nor deliberate radiation hypotension in four cannulated animals tested. The rabbit treated with 200 mg/kg showed ACC with T-wave elevation, one of the two animals treated with 400 mg/Kg experienced acute shock with T-wave elevation, and the rabbit injected with 800 mg/Kg exhibited ACC and T-wave elevation. The average decline in mean aortic pressure averaged for the four rabbits was 58.2% (range: 26.4-71.1, SE=10.7). The 200 mg/Kg rabbit did not have the ear temperature measured, the 400 mg/Kg rabbits showed no jump in ear temperature, and the 800 mg/Kg animal experienced a substantial, sudden rise in ear temperature during the acute blood pressure drop. One cuff monitored animal was injected 30 minutes before TBI with 1.067 mM/kg of cimetidine. This rabbit experienced ACC at about 68 minutes after exposure.

Dexamethasone

Fukuda and Ui using the rabbit showed that hypotension caused by intracisternal injection of calcium ion can be prevented by pre-treatment with cortisone (Fukuda and Ui, 1967). Interestingly, they also found that the hypotensive effects of intracisternal injection with calcium ion could be ameliorated by bilateral cervical vagotomy. Corticosteroids are anti-inflammatory agents which are used in allergic conditions involving edema (Goodman Gilman et al, 1980).

In this study, it was thought that perhaps cortisone might be useful in the treatment of irradiation hypotension. Dexamethasone, a corticosteroid, failed to prevent either acute or deliberate radiation hypotension. ACC developed in 2 out of five rabbits injected with 2 mg/Kg, and in 1 of 2 animals injected with 24 mg/Kg. The decline in mean aortic pressure averaged for all seven rabbits was 44.3% (SE=7). All acute responders showed ECG T-wave elevation as did 1 deliberate responder, and the ear temperature jump developed in all 3 animals experiencing acute hypotension. Perhaps, for the dexamethasone to be effective, it should be injected over several days prior to irradiation.

Digitalis

If radiation directly causes either localized or widespread cardiac damage and impairment of the organ as a pump, then digitalis, which is often used in clinical medicine to boost the ailing heart, might be expected to enhance cardiac performance and thereby raise the blood pressure. However, the 2 rabbits injected with 0.24 and 0.43 mg/Kg digitalis died at 83.7 and 67.7 minutes, respectively, following irradiation. A third rabbit which received digitalis, atropine, and propanolol, died at 73 minutes following exposure. The digitalis was administered an average of 9 minutes before irradiation with no apparent adverse effects.

Endotoxin

Injection with bacterial endotoxin causes sudden and profound shock in dogs and in humans (Lillehei et al, 1964). It was thought that perhaps radiation might cause a release of endotoxin from the lumen of the gut, or it might cause a reaction similar to the shock mechanism elicited by endotoxin. Therefore, bacterial endotoxin was injected in three non-irradiated rabbits to determine if breathing and blood pressure were affected in a manner similar to that seen after whole body irradiation. All three rabbits exhibited labored breathing soon after injection, and in the two cannulated rabbits the mean aortic pressure rose appreciably at about 27 minutes after injection, and remained elevated for roughly 33 minutes (Figure 46). The pressure then declined to 29.8% (range:20.6-38.9) below the lowest pre-injection mean pressure. The lowest pressure was attained about 100 minutes after injection. During much of the time that the pressure was elevated, the pulse pressure was expanded over the previous level. The ECG was unchanged in the one cannulated animal for which it was monitored. In one rabbit the ear temperature started to rise about 2 minutes prior to the increase in aortic pressure, and remained elevated. As shown by Figures 47 and 48, the appearance of the chart tracing after endotoxin was remarkably similar to those recorded with some doubly vagotomized, irradiated rabbits.

IV. Discussion

A. Significance and Characterization of the Acute Response

This report describes a sensitive and practical animal model which is relevant to the radiation hypotension of humans. An important element of this work is the demonstration that the majority of immature, whole body irradiated rabbits exhibited a delayed, precipitous drop in arterial pressure. The sudden pressure fall usually occurred at about 1 hour after exposure, so in terms of timing it is comparable to the early hypotension previously observed for humans and for other warm-blooded species (Painter et al, 1946; Brooks et al, 1956; Shipman et al, 1961; Karas and Stanbury, 1965; Young, 1968; Bruner, 1977). The acute response was elicited using either X-ray or gamma ray doses ranging from 628 to 5,088 cGy. Hence, the data contained herein can be related to hypotension which develops in humans subjected to radiotherapy, and may also at least be considered with respect to the accidental human irradiations which led to death from cardiovascular collapse (Shipman et al, 1961; Karas and Stanbury, 1965; Salazar et al, 1978; Urtasun et al, 1983).

Acute hypotension following TBI has been produced in the rhesus monkey; in this species the hypotension commences either during or immediately after exposure (Chapman, 1968; Young, 1968; Miletech and Strike, 1970; Turbyfill et al,1972; Turns et al, 1971; Doyle et al, 1974; Bruner, 1977;

Alter et al, 1983). There is one report of sudden hypotension in the rabbit. In 1956, Brooks found that New Zealand white rabbits total body exposed to 630 cGy of X-rays exhibited either gradual, or delayed acute hypotension. However no indication was given concerning what proportion of the total number of rabbits experienced the acute response, and no numerical information was given as to the steepness of the acute pressure decline. Also, the analysis of the data is of limited depth, the graphical presentation is unclear, and Brooks neither made particular note of the sudden pressure drop, nor did he mention that the response might be analytically useful. In contrast, the present study is the first, using the rabbit, for which three of the primary, specific objectives were: to produce acute hypotension, to produce it consistently, and to thoroughly characterize the response. In other words, this report describes the discovery of ACC in the rabbit. Also, the distinction between acute and deliberate hypotension has never been before been advanced for any species.

The acute blood pressure fall, as described by the present study, is attractive for several reasons. First, the dramatic all-or-none character of the drop facilitates clean categorization of rabbits as either responders, or non-responders. Thus, changes resulting from various experimental treatments can be assessed with relatively greater certainty and economy; a comparatively large number of adult rabbits, which typically exhibit gradual hypotension, would have to be tested to clearly identify mild, but possibly significant effects. This point is well illustrated by the atropine results of the present study. When 6 mg/Kg of atropine was given the the decline in mean aortic pressure after TBI was 41.4%, compared to 55.4% for untreated controls 40-100 days old. But, the frequency of ACC was reduced from 73% to about 26%--- a marked difference. So, if the atropine tests had been evaluated only on the basis of the depth of hypotension, important information may have been missed. Similarly, the results of double vagotomy are especially striking because acute hypotension was abolished, in addition to the reduction in the depth of hypotension.

A second important feature of acute hypotension in the rabbit is that there is a time lag between irradiation and the onset of the pressure drop. This delay permits experimental intervention and possibly an analysis of the sequence of events leading to the sudden, profound drop.

Another significant aspect of the acute response is that it closely resembles the sudden hypotension which occurs in the rhesus monkey. This is important because the rhesus has been regarded as very relevant to the human (Allen et al, 1960). However, the rabbit model developed in the present study is superior to the rhesus in four respects: (1) the rabbit exhibits a time delay between exposure and the acute drop; (2) the rabbit is much less costly to acquire and maintain; (3) the rabbit is more easily handled; and (4) the rabbit exhibits ACC at 628 cGY, much lower than with the rhesus.

The tail cuff method of blood pressure measurement was intended to reveal whether deliberate hypotension, and in particular, acute hypotension, would occur in non-operated rabbits after irradiation, or whether one or both types of pressure reduction had arisen only because animals had been subjected to anesthesia and surgery prior to exposure. Although the cuff method did not provide continuous readings during each run, and only recorded systolic pressure, the results from this approach proved that the irradiation hypotensions of anesthetized, cannulated rabbits are very similar to those of non-operated rabbits. The acute response occurred in just over 50% of the group of cuff monitored rabbits subjected to TBI with between 950 and 2,850 cGy. Also, ECG T-wave elevation and the sudden ear temperature rise, both of which frequently accompany the acute drop, were observed in non-operated acute responders. The onset time of acute hypotension on average was 6.6 minutes later for the cuff monitored group, but this difference is not great, and seems in part to have been due to limitations of the cuff technique (Appendix I). Noninvasive blood pressure measurements following whole or upper half body irradiation, have not been previously reported for any species other than humans and rats. However, the irradiated rat does not exhibit ACC, only relatively shallow deliberate hypotension, and in one test the tail cuff method indicated hypotension, while invasive arterial measurements did not reveal any hypotension in this animal after a comparable irradiation dose (Phillips and Kimeldorf, 1963).

A major emphasis during this project was to thoroughly characterize acute hypotension. Consequently, the number of animals tested in this study is greater than the number of animals used in all other studies of irradiation hypotension combined--- acute hypotension was produced in, and analyzed using hundreds of rabbits during the course of this study.

This report demonstrates that the frequency of ACC depends on age. The highest proportion of acute responders is in the 40 to 100 day group, while older animals tend to exhibit deliberate hypotension. This is the first such demonstration in the radiation physiology literature, and this finding is a probable explanation as to why previous investigators have almost exclusively reported gradual (albeit profound) pressure reductions after TBI of the rabbit; they must have used young adult and/or mature rabbits.

A relatively broad range of physiological parameters was measured and recorded in this work. The ear temperature was found to rise sharply at the time of ACC, which suggests that peripheral vasodilatation may be at least partly responsible for the hypotension. The measurement of skin temperature as a method of disclosing peripheral vasodilatation (or erythema) has never before been used in radiation studies, and the detection of peripheral vasodilatation in conjunction with blood pressure measurement after irradiation has never before been attempted.

The doses employed in this study ranged from 628 cGy to 5,088 cGy whole body, and the majority of exposures were between 900 and 1,500 cGy. The doses within the latter range are comparable to exposures used in whole, or upper half body radiotherapy of cancer patients. Therefore, at least in terms of radiation dose, this project may be related to the hypotension of humans which follows radiation treatment. However, caution is necessary when comparing the present work to the cases of fatal cardiovascualar collapse resulting from nuclear plant accidents at Los Alamos and Rhode Island. The basis for reservation is that the victims were exposed to several thousand centiGray of mixed gamma-neutron radiation, while the rabbits in the present study were exposed to 1.2 MeV gamma rays or to 6 MeV X-rays. Neutrons have a relative biological effect (RBE), which is roughly three times that of photon radiations. The cardiovascular effects of neutron radiation have not been tested in the rabbit, but Young and Tyler (1982) found in the rhesus monkey, that neutron radiation more effectively elicited emesis than did the same doses (in terms of cGy) of gamma rays. Nevertheless, prompt emesis did in fact occur in both gamma and neutron irradiated monkeys. In the present project, the occurrence of ACC and deep deliberate hypotension in rabbits exposed to 5,088 cGy of gamma rays is encouraging. This is because this dose is about the same as the gamma equivalent received by the Argentinian nuclear plant worker referred to in the Introduction. The US Nuclear Regulatory Commission information notice indicated that this man was accidentally exposed to 1,400 cGy of fast neutrons and 500 cGy of gamma. However, the dose reports for the amounts of neutron and gamma radiation this individual was exposed to vary, but from one of the estimates, it has been reckoned that this man received a gamma equivalent bone marrow dose of roughly 5,000 to 6,000 cGy. This would put this case in the same class as the Los Alamos and Rhode Island accidents.[22] The victim exhibited at least some of the symptoms of acute radiation sickness, exhibited nervous disorders, and died 49 hours after exposure, apparently from inflammation of the lungs (Jordan, 1983).

The present report also shows that ACC can occur over a wide range of dose rates, and with a variety of different irradiation sources. The Gammacell 220 produced a non-uniform pattern of dose deposition, while the 6 MeV X-ray arrangement provided a very homogeneous exposure; with both approaches the frequency and character of ACC was the same. Low dose rates generated by the Theratron 80 were at least as effective at triggering ACC as was the very high dose rate generated by the Gammacell early in the project (Table VI). However, the time of onset of ACC varied between the three radiation sources. With the Theratron 80/780, acute hypotension developed later (Table VI). This may have been due to the lower dose rates produced by the Theratron units. Low dose rate lengthens the period of time needed to deliver a significant dose, so that the onset of ACC might be later with respect to the beginning of the irradiation. Also, it may be possible that the various dose rates employed differ in terms of their biological effects.

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[22] This estimate was generously proffered in a personal communication by Dr. C. Lushbaugh of the Oak Ridge National Laboratory, in Oak Ridge, Tenn.

B. Interpretation of Experimental Results

Selective irradiation and selective shielding of the heart region were attempted after it was clear that ECG T-wave elevation (Plate 13) was often observed during acute hypotension, and sometimes during deep deliberate hypotension, and that cavity potentials occasionally developed shortly after the acute pressure drop. Cavity potentials are associated with the death of a mass of ventricular muscle, while T-wave elevation represents myocardial ischemia. Hence, the objective was to determine if radiation directly damaged the heart, thereby causing cardiac dysfunction and resultant hypotension. But, it was also recognized that the ECG manifestations could arise either from direct radiation effects on the heart, or from a reduction in coronary perfusion stemming from systemic hypotension originating outside the heart. The results clearly show that the heart region is an important target; the level of hypotension produced by selective irradiation, and the degree of benefit derived from shielding, are substantial, considering the relatively small volume of the body involved. Chapman (1968) found that irradiation of the trunk alone elicited hypotension which was almost identical in terms of depth and character to that produced by the same doses of TBI. However, the results of the present study do not necessarily mean that the heart is the critical target, as parts of the lungs, nerves and neural receptors, major blood vessels, and the blood, are also exposed in heart region irradiation. The entire blood volume, and portions of all the aforementioned structures, are also irradiated during heart shielding with whole body exposure. Later experiments from this study which did not support the hypothesis that heart damage alone is responsible for radiation hypotension in rabbits are referred to further on in the Discussion. The heart has long been regarded as being quite radioresistant (Gordon et al, 1924; Emery and Gordon, 1925; Ajisaka, 1936; Leach, 1942; Warren, 1942). However, Ahrens observed with the light microscope that mitochondrial blebbing occurred in cultured (in vitro) chick cardiac cells at one hour after exposure to 2,400 cGy of X-rays cGy/minute (Ahrens, 1972). Several workers have found evidence of cardiac damage appearing in animals within hours to months after a one to several thousand cGy to the heart or chest area (Caster et al 1957, Lamberts and deBoer, 1964; Phillips et al 1971). Notably, Fajardo demonstrated in the rabbit, extensive cardiac fibrosis which was distinct by about 70-75 days after a single, 2,000 cGy exposure of the heart (Fajardo and Stewart, 1970; Fajardo and Stewart, 1973). He also found that a widespread heterophil exudate occurred in the myocardium, endocardium, and in the heart blood vessels, commencing at about 6 hours, and lasting about 48 hours after exposure. This reaction is termed "Acute Radiation Pancarditis" (Fajardo and Stewart, 1970; Fajardo and Stewart, 1973). In humans fibrosis has developed within months of several chest exposures of 1,100 cGy per week (Fajardo et al, 1968). Catterall has cautioned that radiotherapy to the thorax may pose a risk of cardiac damage in patients with preexisting heart ailments (Catterall, 1960). Few studies have been reported in which functional changes in the heart were recorded within hours of cardiac irradiation. Fulton found that by the fourth day after TBI of golden hamsters with 1,500 cGy, the ECG T-wave was elevated (Fulton and Sudak, 1954). Caster et al (1957) found that within 2 hours total body exposing rats to 700 cGy, there was in some cases an increase in the T height of the central chest lead. The present study is the first example of hypotension with ECG T-wave elevation, developing within hours of irradiating the heart region. Of particular note is the fact that the doses employed were relatively low, 1,200 and 2,000 cGy.

It is possible that direct radiation damage to that heart occurs, and that that heart failure leads to the shock. The cavity potentials, or "Q-waves" which were in a few instances recorded, would be caused by direct damage to the heart, but not by reduced coronary perfusion.[23] Also, the decline in ear temperature after irradiation suggests that sympathetic outflow may be occurring after TBI, to help maintain the central pressure in the face of a failing heart. The ear temperature rise at the time of ACC may represent collapse of symapthetic tone--- this collapse may cause ACC. Damage to the left ventricle could cause the filling pressure to increase, which in turn would raise the pulmonary arterial pressure. Thus, the pressure in the right ventricle could rise before ACC. However, double vagotomy abolished ACC, and would not be expected to markedly assist the sympathetic system. Also, selective irradiation of the heart with both vagi cut, resulted in relatively mild hypotension, indicating that cardiac damage, if it occurs, does not by itself cause ACC. Furthermore, treatment with alpha and beta blockers together, would be expected to oppose any sympathetic support of the circulatory system that may develop early after radiation induced cardiac damage. However, this drug combination did not hasten the onset of ACC, meaning that critical sympathetic support probably did not occur. Also, significant damage to the myocardium would be reflected by a rise in the left ventricular end diastolic pressure (LVEDP).[24] But, in this laboratory, measurement of LVEDP in two TBI rabbits (one apparently showing deep deliberate hypotension) uncovered no significant change in that parameter after irradiation. Nevertheless, in another TBI rabbit, the LVEDP may have been markedly elevated just after ACC. The appearance of Q-waves in some TBI rabbits is of interest, but it should be noted that Fajardo and his colleagues carefully examined the hearts of rabbits which had received a single cardiac dose of 2,000 cGy. There was no mention of infarcted areas on these hearts being evident within 48 hours of irradiation (Fajardo and Stewart, 1970; Fajardo and Stewart, 1973).[25] Thus, as indicated earlier, it is not known whether changes within the heart after TBI are due to direct radiation damage, or arise from other, systemic changes. Furthermore, it is possible that any histological changes which might develop in the heart may be secondary to hypotension and/or neural aberrations originating outside this organ. In view of this point, it was decided to defer extensive histological examination of irradiated hearts until after it was determined whether any other structures contributed materially to irradiation hypotension. Indications in the literature of neural effects following radiation exposure were sufficient to warrant testing vagotomy, and atropine, with whole body irradiation.

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[23] Personal communication, Dr. R. E. Rossall, Department of Cardiology, University of Alberta.

[24] Personal communication, Dr. R. E. Rossall, Department of Cardiology, University of Alberta.

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[25] It should be noted for the heart irradiation/shielding experiments of the present study, that the position of the heart with respect to the field marked on the lucite box was not rechecked after the rabbit was moved from the treatment simulator to the irradiation unit. Therefore, it is possible that the position of the rabbit may have shifted just prior to the actual heart irradiation/shielding, resulting in an incorrect exposure. This might account for the fact that one of the heart shielded rabbits exhibited ACC. Ideally, an X-radiograph should have been taken with a Cerrobend block against the marked field, after the box was positioned on the irradiation device treatment bed. This would have revealed what the position of the heart was, with respect to the marked field, immediately prior to exposure.

Rabbits which were bilaterally vagotomized did not experience the acute pressure drop, and the depth of hypotension in them was about half of that recorded with non-vagotomized animals. These results imply a vagal role in acute and deliberate radiation hypotension. But, they also must be regarded with care, because of the stress imposed by double vagotomy. However, single vagotomy is much less stressful (rabbits do not die from this procedure)[24], and the extent of radiation hypotension is not as great as that in non-vagotomized animals. The effects of atropine injection are generally consistent with the vagotomy results. The reduction of radiation hypotension with atropine was mild, but the incidence of acute hypotension was strikingly diminished. Therefore, despite reservations about double vagotomy as an experimental approach, the possibility of an important neural role in radiation hypotension cannot be ignored. However, the nature of this putative participation cannot be deduced in complete form on the basis of the present results. The rabbit vagus contains parasympathetic and sympathetic afferents and efferents, and of course all are severed with vagotomy, so the identity of the neural pathway involved is not known. The reason why atropine only marginally affects the depth of hypotension also is not known. Perhaps parasympathetic cholinergic, sympathetic adrenergic, and other types of nerve fibres all participate in radiation hypotension. If this is true, atropine would only exert a partial effect by blocking cholinergic transmission. In any event the atropine produced its effects by blocking acetylcholine receptor sites outside the central nervous sytem---this is clear because atropine methyl bromide (AMB) was used for almost all the atropine tests, and this form of atropine does not cross the blood - brain barrier. Furthermore, AMB would be expected to mostly impede efferent cholinergic transmission, so it seems that some type of efferent activity may be involved in ACC. A key question which arose following the double vagotomy and atropine tests, related to why these treatments changed the course of the hypotension. It was considered possible and at the time of this writing is to some extent, still regarded as possible that changes in vagal flow, if they occur, are not connected with radiation hypotension. Bilateral vagotomy and high doses of atropine may affect the incidence of acute hypotension, and the depth of hypotension, by disrupting the rabbits' physiological condition, not because they blocked vagal signals which normally triggered radiation hypotension. Therefore, a direct measure of vagal traffic in irradiated animals was attempted, to determine if whole body radiation exposure did induce a change in the electrical activity of the vagus.

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[24] Rabbits with one cervical vagus cut have survived normally indefinitely (Farber, 1940).

In rabbits, recordings of transmission in the vagus and in single vagal fibres, have been achieved using hook and microelectrodes respectively, with the animals fully anesthetized. Only one report is available (in the open English language literature) of the measurement of vagal nerve activity in irradiated rabbits. This is a review which summarizes two Russian reports (Livanov and Biryukov, 1962). These appear to have comprised time based recording of the amplitude of voltage potentials generated by the vagi of anesthetized rabbits. Records were made before and after whole body exposure to 1,000 cGy of X-rays. For the present study, vagal monitoring was attempted in conscious rabbits, because all previous work had been performed using conscious animals, and because anesthesia might distort or mask any radiation induced changes in vagal transmission. Consequently, microelectrodes could not be used. The basis for this rests with the fact that microelectrodes must be placed extremely near a nerve fibre, as the potentials produced by the fibre fade very quickly in the extracellular fluid. Since a microelectrode penetrating the cervical vagus cannot be secured to any immobile, solid structure (e.g., the skull), the slightest body movement would jerk the electrode far out of position (Schad and Seller, 1975, Stein et al, 1975). Another drawback of microelectrodes in this application might arise because the cervical vagus of the rabbit contains about 23,000 individual fibres (Evans and Murray, 1954). Following whole body irradiation, some vagal fibres might remain inactive or exhibit no change in activity, while others perhaps sustain changes in transmission. If this is the case, the vagus would possibly have to be probed in many rabbits to reveal any consistent change associated with radiation. On the other hand, the silastic nerve cuff can operate very effectively in conscious animals. This device in some respects is similar to the hook electrode system used for neural recording in anesthetized animals. With the hook electrode approach, metal hooks are placed in contact with, and partly around a nerve, and the surgical site is filled with paraffin oil for electrical insulation. This system measures the moment-to-moment sum of all the fibre firings within the nerve. The nerve cuff in essence contains three hook electrodes, it measures bulk vagal activity, and is made of a silicone rubber cylinder which performs the function of paraffin oil. Since the cuff picks up the relatively large electrical signals generated by an entire nerve, the need for close contact is obviated. Hence, movement of the animal, and the associated movement of the cuff relative to the nerve has little, if any effect on recording. The silastic wall insulates the electrodes from EMG interference, and EMG voltages entering the ends of the cuff are negated by a common mode rejection circuit fed by the three electrodes. The cuff system is designed to minimize EMG contamination of the neural recording, but some EMG signals are picked up and amplified by the recording system.

The objective with the present study was to ascertain whether a change in the extent and/or pattern of vagal activity develops in fully conscious 40-100 day rabbits after TBI. Since all pre- and post-irradiation blood pressure recording done in this project involved only conscious rabbits, it was desirable to monitor vagal activity in conscious animals so that the nerve results would be more directly relevant to the pressure tests. However, time domain recordings derived from conscious rabbits comprised an array of frequencies and were very complex, so that discerning changes and trends was most difficult and could not be performed with confidence. Consequently a power spectrum analyzer was used. This instrument essentially contains a series of filters which partition a comingling of signals into pre-set, discrete frequency ranges, or bands, each of which possesses a central representative frequency. The total voltage occurring approximately at each central frequency is measured, and voltage versus frequency is displayed as a moment-to-moment bar graph on a CRT screen. This type of display is termed a power spectrum, or frequency domain, and renders even relatively small changes in voltage amplitude within a defined frequency spectrum, easily recognizable.

When rabbits were injected with sedating or lethal doses of pentobarbital, the lefthand curve of the vagal power spectrum vanished. This finding, together with the fact that the lefthand curve resided at relatively low frequencies, indicates that this feature most probably was produced by voluntary muscle noise. The persistence of the righthand curve after pentobarbital, and the presence of its voltage amplitude peak at around 1600 Hz, suggest that this curve resulted from vagal transmission. So, it appears that at least in some cases the cuff system successfully detected vagal nerve transmission. Furthermore, 11 of 13 rabbits showed at least a slight elevation of the neural peak of the vagal power spectrum over the pre-irradiation level. This means that radiation elicited a rise in the total electrical activity of the vagus. Since the firing of individual fibres occurs on an all-or-none basis, and the amplitude of fibre action potential is fixed, the power spectrum results imply that radiation either caused an increase of the frequency of firing in at least some of the vagal fibres, and/or the number of active fibres had risen. The results are in agreement with the Soviet report (Livanov and Biryukov, 1962) of higher vagal potentials in rabbits after whole body exposure, and lend support to the possibility that the vagi participate in radiation hypotension. However, the vagal monitoring tests do not reveal whether the putative increase in voltage amplitude is a/the cause of irradiation hypotension, or a consequence. Also, the work does not indicate whether the voltage amplitude rise is due to increased efferent activity, increased afferent activity, or due to increased transmission in both directions. Still, these results, together with the findings of double and single vagotomy, and of atropine, suggest that the vagi somehow participate in irradiation hypotension, and further, more specific neural measurements would not be unwarranted. The cuff work is the first neurophysiological measurement relating changes in the nervous system to radiation induced hypotension; this has not been done before. However, the nerve cuff data must be regarded with a good deal of caution, as the post-exposure increment in neural peak voltage was not especially large, and thus may have resulted from EMG noise. The height of the EMG curve was increased following irradiation, and in some cases what appeared to be EMG potentials caused the oscilloscope bars, or "steps", to rise substantially in the mid-region of the power spectrum. The higher frequency harmonics of all the EMG noise may have spilled into, and elevated the neural curve voltage levels. Fortunately, the liklihood that this took place in most of the recordings is not great. This is because the EMG voltages usually resided in frequency bands which were far from the frequency band containing the neural peak, and were even farther from the descending limb of the neural curve. Both these components of the neural curve were elevated, and spillover voltages from EMG signals would probably fade away well before reaching higher frequency bands within the neural curve.

Double vagotomy is a crude method by which to reveal participation of the vagi in irradiation hypotension, because doubly vagotomized rabbits suffer a considerable stress which by itself leads to death within about 24 hours of surgery (Farber, 1937). Cutting both vagi abolishes autonomic control of the flow of blood through the lungs, causing pulmonary edema (Farber, 1940). Bilaterally vagotomized rabbits experienced dyspnea and could not swallow as soon as the depth of general anesthesia had lessened following surgery. One nonirradiated rabbit died overnight from this procedure.

Therefore, the interpretation of any results obtained using doubly vagotomized rabbits must be qualified by the fact that seriously abnormal animals were involved. Also long-term post-irradiation survival and blood pressure studies are not possible with doubly vagotomized rabbits. Therefore, reversibly interrupting vagal transmision by cooling the nerve was attempted. The effects of double vagotomy on radiation induced cardiovascular collapse should be attainable by cutting one vagus, and interrupting the activity of the other during, and for at least 90 minutes after exposure. The cooled vagus could then be allowed to rewarm and renew transmission so that the rabbit would not die from respiratory distress--- unilaterally vagotomized rabbits experienced no obvious difficulty breathing. The vagal cooling device, if small enough, could be placed on the vagus along with the nerve cuff. Such an arrangement could be used to provide further evidence for the identity of the putative vagal curve in power spectrum recording; if in several rabbits the vagus were cooled to a point assuring cessation of transmission, and the righthand profile vanished, this would constitute a strong indication of vagal origin for this curve. Interruption of vagal activity at various times after irradiation might help discern the temporal relationship between vagal transmission and acute and deliberate hypotension. Also, the importance and timing of afferent and efferent vagal signals on acute and deliberate radiation hypotension might be examined, since efferent and afferent flow are blocked at different temperatures (Partridge, 1939; Phillipson et al, 1973).

Double vagotomy and heart shielding were applied together in an attempt to eliminate post-irradiation hypotension in 10 rabbits. Combining these two treatments reduced the average depth of the decline in mean pressure to only 9.9% (range: +7 to -32.2) over the first two post-irradiation hours. Comparable protection against the hypotension has been claimed for epinephrine and norepinephrine, administered to the rhesus monkey and the rabbit (Painter et al, 1946; Miletech and Strike, 1970; Turns et al, 1971). With both species the doses needed were high. The monkeys all died within hours of TBI, and in one report the norepinephrine treated monkeys died sooner after TBI than non-treated monkeys (Turns et al, 1971). This was probably because norepinephrine maintains central pressure at the expense of peripheral tissue perfusion (Turns et al, 1971). (There appears to be no information on the post-TBI survival of adrenalin treated rabbits.) So, norepinephrine, and likely epinephrine, are not satisfactory therapy for radiation hypotension. In the rhesus, H1 receptor antihistamines given alone or with H2 agonists, abolished early, acute hypotension. But, deliberate hypotension was somewhat mitigated, not abolished.[27] In this project, double vagotomy with heart shielding abolished ACC and greatly reduced deliberate hypotension for up to two hours post-TBI.

The importance of the results from experiments combining double vagotomy and heart shielding, is not that these two treatments together constitute a practicable therapy for humans; clearly this is not so. The significance of this work is that it has identified a specific part of the nervous system and the heart region, as critical in radiation hypotension, and that treatments involving these structures can be very effective at reducing the hypotension. Hence, further research can focus on the vagi and the heart to formulate specific therapies which might be potent and practical. The reason(s) why heart shielding together with double vagotomy so effectively diminished radiation hypotension is/are not known. Nevertheless, some inferences may be drawn about the liklihood that certain body targets, and proposed mechanisms, are major factors in radiation hypotension. First, since the combination of heart shielding and double vagotomy is more protective than double vagotomy alone, heart shielding and double vagotomy may affect different targets/systems. However, this is by no means certain as the extent of hypotension does not differ by a statistically significant margin (Student's t-test) between the heart shielded group and the double vagotomy with heart shielded group. Secondly, the results definitely do not support the idea that histamine acts directly on the peripheral vasculature, eliciting extensive vasodilatation and hypotension. If this mechanism were important, then double vagotomy with heart shielding would probably not be very effective. This reasoning is made more compelling in light of the fact that in rabbits the blood is a particularly rich source of histamine (Code, 1937). Also, it has been found in rats at least, that the small intestine is a site which contains a relatively large quantity of histamine (Gustafsson et al, 1957). Both the blood and the gut are exposed during heart shielding. Perhaps, peripheral vasodilatation caused by histamine may be responsible for the occasional, relatively mild radiation hypotension seen in heart shielded, bilaterally vagotomized rabbits. And, an important role for histamine in radiation hypotension is not precluded. It is possible that radiation induced histamine release within the central nervous system causes serious disturbances of function. Histamine is formed in the mammalian brain, and histamine injected into the brain ventricles of conscious cats increases respiration, induces vomiting, sedates, and produces muscular weakness (Feldberg and Sherwood, 1954; White, 1964). It should also be considered that perhaps the irradiated heart is rendered especially sensitive to histamine.

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[27] In one report, rhesus monkeys injected before TBI with 20 mg of the H1 agonist chloropheniramine, had the mean arterial pressure begin to gradually decline shortly after TBI, and by about 120 minutes after exposure it was 92% of the pre-irradiation level. The monkeys treated with 40 mg of chloropheniramine had the mean arterial pressure decline to 68% of the pre-exposure level, by 100 minutes after TBI. Five animals were tested in each group (Doyle et al, 1974). In another report, rhesus monkeys treated with H1 and H2 antihistamines had the mean arterial pressure decline from 117 mm Hg to 78 mm Hg. In this case 4 animals were tested (Alter et al, 1983).

The outcome of bilateral vagotomy plus heart shielding strongly implies that extravasation of the blood, due to direct radiation damage to the peripheral vasculature, if it occurs, is not sufficiently extensive to result in substantial hypotension. If marked extravasation was present after irradiation, then double vagotomy with heart shielding would not have been effective. Also, the post-irradiation hematocrit was unremarkable, even for blood drawn shortly after the acute pressure drop. This indicates that plasma loss to the tissues either did not take place, or was minimal. Several investigators have suggested that appreciable damage to the skin vasculature is present within hours of local irradiation (Rigdon and Curl, 1943; Painter et al, 1946; Jolles and Harrison, 1966; Eassa and Casarett, 1973). Such an event may at least in part depend on the penetrating power of the radiation employed (Pizzarello and Witcofski, 1967). Higher kilovoltage X-rays would be less likely to deposit a substantial proportion of their energies in superficial body layers, while lower kilovoltage X-rays would impart a relatively higher skin dose.[28] Particle radiations, due to their high rate of linear energy transfer (LET), would be more likely to cause erythema than photon radiation. In any case, some gamma exposed rabbits in the present study seemed to exhibit skin erythema of the nose, and the ear temperature rose sharply during acute hypotension. However, the post-irradiation ear temperature profile was quite altered when alpha adrenergic blocking agents were administered, so the temperature jump likely stemmed from neural events.[29] Nevertheless, vascular damage may form an important part of the mechanism of radiation hypotension, viz., it is possible that changes in the capillaries in various organs, such as the brain, heart and lungs, are important.

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[28] None of the references listed here dealing with the skin reaction involved X-ray energies greater than 280 kv, and three of the studies used so-called "superficial" X-ray units. The energies with these units were 86 or 100 kv.

The protection provided by double vagotomy and heart shielding, precludes the possibility that if only the blood and the lungs are irradiated, deep hypotension can develop. The entire blood volume and much lung tissue are exposed during heart shielding. Thus, if irradiation of one or both targets alone resulted in marked hypotension, then double vagotomy with heart shielding would probably not be effective. The results of heart irradiation of doubly vagotomized rabbits are significant with respect to lung sensitivity. The hypotension in these animals was relatively shallow, contradicting the possibility that heart shielding was effective because a highly sensitive area of the lungs was protected. A key cautionary possibility is that radiation damage to the lungs and/or the blood may cause severe hypotension only when the entire body is exposed and/or one of the vagi is intact.

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[29] The alpha adrenergic results are not conclusive as few rabbits were tested.

The results of selective irradiation/shielding of the heart immediately raise the question of whether direct radiation effects on the heart result in cardiac dysfunction, and a resultant drop in blood pressure. Considering the relatively shallow hypotension which occurred in bilaterally vagotomized rabbits which were heart region irradiated, it seems that exposure of the heart alone causes little, if any hypotension. Furthermore, selective heart irradiation with the vagi intact elicits significantly deeper hypotension than when both vagi are cut. This implies that the basis for heart region sensitivity may not be that the heart muscle is rendered incapable of functioning effectively, but that other structures in the area of the heart, perhaps various neural receptors and/or the lungs, are affected by radiation, and that these interact with the vagi to produce hypotension. Or, it may be that the irradiated heart is very vulnerable to vagal stimulation, and/or events or substances originating outside the heart region during whole body exposure. The marginal hypotension seen after exposure of doubly vagotomized, heart shielded rabbits cannot be due to direct radiation effects on the heart.

Vagal outflow could conceivably elicit a blood pressure decline by three different mechanisms, operating either alone or in any combination. First, if the efferent parasympathetic vagal fibres are active following irradiation, they would liberate acetylcholine (ACh), which would prompt reduction of the force and the rate of cardiac contraction.

If sufficient ACh is released, hypotension can result. However, the drop in heart rate which ought to accompany vagal effects on the heart was rarely observed; in many cases the heart rate increased just prior to and during the acute drop, as though responding to sympathetic stimulation.

The second mechanism by which vagal outflow can lead to hypotension is by stimulating extensive constriction of pulmonary blood vessels. Parasympathetic cholinergic fibres, sympathetic adrenergic fibres, and various histaminic fibres leave the thoracic vagal trunk and supply the lung vasculature and smooth muscle of the airways. Marked constriction of the pulmonary vasculature can impede the flow of blood from the right ventricle through the pulmonary artery; the latter is quite muscular in rabbits. This can cause a syndrome termed "cor pulmonale"; the right ventricle fails because of the stress of forcing the blood through the constricted pulmonary circuit. This is rapidly following by left ventricular failure, due to the inadequate return of oxygenated blood. If after irradiation vagal outflow elicits pulmonary vasoconstriction, along with one or both of, depression of cardiac function and peripheral vasodilatation, the resultant hypotension could be severe. Von Euler found that electrical stimulation of the cut cervical vagus in anesthetized rabbits elicited bronco-constriction and a sharp rise in pulmonary artery pressure (PAP), and that atropine blocked this response (Von Euler, 1932). He also found that injection with acetylcholine caused broncho-constriction and a rise in PAP, but that adrenalin produced a small rise in PAP. Ettinger and Hall (1935) found that injection of ACh in the rabbit caused a drop in aortic pressure, constriction of the pulmonary blood vessels, and a rise in PAP, while adrenalin had a feeble effect. For the present study, it was reckoned that if irradiation caused the resistance to pulmonary blood flow to markedly increase, then the pressure within the pulmonary artery and the right ventricle should rise appreciably. Langille found that occlusion of the pulmonary artery in anesthetized rabbits resulted in distinct elevation of the right ventricular pressure (Langille, 1975). Hence, for the present study it was decided to record right ventricular pressure from TBI rabbits.

The results of this study demonstrate that the pressure within the right ventricle rises substantially in the majority of 40-100 day old, cannulated rabbits which are whole body irradiated. [30] Animals experiencing the acute arterial pressure drop displayed a rapid increase in right ventricular pressure, which in three cases commenced by an average of 4.3 minutes (SE=1.25) prior to the acute drop, and reached roughly 90% of its peak value at the onset of acute hypotension. In the two steep deliberate responders, the right ventricular pressure began to rise before the onset of the steepest phase of the arterial pressure decline. This temporal relationship indicates that the rise in right ventricular pressure causes the drop in aortic pressure, rather than the opposite. The results suggest that acute and steep deliberate hypotension to an important extent may result from some obstruction to pulmonary flow which occurs within the pulmonary circuit. The timing of the right ventricular pressure jump in acute, and in some deliberate responders, is consistent with the outcome of selective heart irradiation in doubly vagotomized rabbits. The results of both experiments contradict the notion that direct radiation damage to the heart, is alone sufficient to elicit deep hypotension after TBI. Radiation damage to the heart may be an important factor in radiation hypotension, but left ventricular failure, if it occurs, develops after the right ventricular pressure rise. The results of double vagotomy, atropine, vagal recording, and heart irradiation with double vagotomy, suggest that the post-irradiation increase in right ventricular pressure may be triggered by vagal outflow. An important future experiment in this context would be to measure right ventricular pressure after whole body exposure of doubly vagotomized rabbits, or preferably, of animals in which both vagi are inactivated by cooling.

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[30] Note: recent work in this laboratory has shown that at least to some extent, the increase right ventricular pulse amplitude following TBI is due to labored breathing. The right ventricular pressure is superimposed on a continuous sine pattern created by inspiration and expiration. This has been revealed by operating the chart recorder at very high paper feed rates. However, the increase in amplitude does not distort the value of the mean pressure, and this has been shown to rise after TBI (Table IX).

Two studies provide evidence which agrees with the cor pulmonale hypothesis for radiation hypotension. Korosower et al intravenously injected New Zealand white rabbits with Iodine 131 labelled albumin, and irradiated the animals over the chest with 3,000 cGy of 280 kv X-rays at 200 cGy/minute (Korosower et al, 1971). By about two hours after irradiation the level of radioactivity within the lungs had fallen well below the pre-irradiation condition, indicating a drop in lung perfusion by the blood. Also, angiography of the lung vasculature using intravenously injected contrast medium, revealed that the flow of blood through the pulmonary artery had declined. The authors did not indicate whether any signs of radiation sickness developed. A very informative future experiment might be to use radioisotopes to determine the degree of lung perfusion in whole body - irradiated rabbits, for which arterial and right ventricular pressures are monitored. Were a reduction in lung perfusion to be observed, the timing of this event with respect to the right ventricular pressure rise in acute responders, would be of major importance. For example, if a substantial, rapid drop in lung perfusion coincided with the right ventricular pressure increase, and preceded acute arterial hypotension, then it could be concluded that acute and steep deliberate radiation hypotension result from obstruction of pulmonary blood flow, and subsequent right heart failure. If such a process were shown to occur, then possibly both vagi could be cooled during the right ventricular pressure jump, but before the acute drop. Any reversal of the right ventricular pressure increase, a resumption of pulmonary perfusion, and the absence of acute hypotension, would be powerfully indicative of one major way by which the vagi participate in radiation hypotension.

The second study which provides evidence consistent with the cor pulmonale hypothesis is presented by the pathology reports of accidentally irradiated nuclear workers at Los Alamos and Rhode Island, who died from cardiovascular collapse (Shipman et al, 1961; Karas and Stanbury 1965). The right ventricle of the Rhode Island victim was dilated, with interstitial hemorrhage present. The left heart was entirely normal. The pathologist who autopsied the Los Alamos patient stated that the cause of death was "clinical right heart failure". The right ventricle was dilated, with some interstitial blood, while the left heart was normal. In both cases, the lungs were mildly congested.

It has been found in humans that head injuries can lead to pulmonary edema, dilation of the right ventricle, and death within minutes. Simmons et al (1969) reviewed 56 cases in which soldiers had sustained head injuries (usually gunshot wounds) and had died within several days. The autopsies disclosed that those who had died within minutes of the trauma had edematous lungs, and a few of these individuals had a dilated right ventricle. These findings imply that neural outflow can lead to changes in pulmonary physiology with dilation of the right heart. However, the brains of the

Los Alamos and Rhode Island accident victims were normal microscopically, and the lungs were not described as particularly edematous.[31] But, obstruction to pulmonary flow can be due to neurally triggered pulmonary vasoconstriction, and does not necessarily have to arise from edema. But, functional changes in the brain after irradiation with doses below those which might be expected to cause obvious microscopic damage has been found in animals (Gerstner et al, 1955; Brooks, 1956; Caster, 1958; Gangloff, 1959; Haley, 1960; Barnes, 1967). In one study, head irradiation of rhesus monkeys with 2,500 cGy caused the mean aortic blood pressure to decline from 119 mm Hg to 103 mm Hg; the decline was shown to be statistically significant (with _P_ less than 0.005) (Chapman and Young, 1968). TBI of rhesus monkeys with 3,000 cGy has been reported to result in rapid death, with no cerebral edema, but with meningeal congestion and pulmonary edema (Wilson, 1960). Ducker and Simmons (1968) demonstrated using dogs and monkeys, that when an inflatable balloon was placed against the brain and pressurized, 20% of the subjects developed pulmonary edema. Increased venous pressure was invariably associated with the pulmonary edema.

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[31] However, for both cases the lungs were somewhat edematous, as were the brains and meninges. The cerebral edema was in one case attributed to right heart failure. If the meningeal and/or cerebral edema were not consequences of right heart failure, and were present for some time before death, possibly pressure on the brain was created, resulting in neural dysfunction, neurally induced pulmonary vasoconstriction, and cor pulmonale. On the other hand, the pulmonary edema and congestion may have been due to direct radiation effects on the lung, and may have been sufficiently extensive to significantly impede pulmonary blood flow, and cause right heart failure.

Caster et al (1957) found that at about 10 days after TBI with 700 cGy, rats had marked edema of the heart and lung. The animals also exhibited an increase in the plasma volume of various structures within 3-12 hours of exposure. But, in the rabbit the head must be exposed to several thousand cGy in order for deep hypotension to develop (Gerstner et al, 1956). In the present study head irradiation with 1200 cGy may have elicited mild hypotension; one rabbit had a total decline which was about 3.8% below the lowest pressure for sham exposed rabbits. It is of interest that an Argentinian nuclear plant worker who died 49 hours after accidental exposure, exhibited some nervous disorders, and severe inflammation of the lungs. The lungs may have been "inflamed" due to direct, or other effects--- possibly neural, as with the soldiers described above (Jordan, 1983).

It may be the case that the pulmonary vasculature is stimulated to constrict by histamine released from radiation damaged tissues. A review by Daley and Hebb (1966) describes how several workers have found that histamine powerfully constricts pulmonary arterial smooth muscle and vessels; there are histamine receptors in the lung, and histamine has been postulated as one of the neurotransmitters for pulmonary vagal fibres (Daly and Hebb, 1966). However, if histamine causes obstruction to pulmonary blood flow and consequent right heart failure in irradiated rabbits, it is difficult to reconcile this with the protection afforded by the combination of heart shielding and double vagotomy.

Still, at least some pulmonary vasoconstriction may be induced by histamine after irradiation, and this may be the basis of the hypotension which does occur in doubly vagotomized, heart shielded animals. Therefore, if histamine were to act on the lungs of irradiated rabbits, it would exacerbate radiation hypotension in animals which were whole body exposed without heart shielding, and which had both vagi intact.

It is possible however, that radiation may damage the lung vasculature directly, leading to some degree of pulmonary edema. Damaged lungs, and possibly a damaged heart, may interact with the vagi to produce deep hypotension following irradiation. The lungs are sensitive to radiation, and lung damage following radiotherapy to the chest has often been recorded (Warren and Spencer, 1940; Whitfield et al, 1963; Van Dyk et al, 1981; Urtasun, 1983; Urtasun et al, 1983). Clinical UHBI often involves doses of 600 cGy or greater, and radiation pneumonitis of the exposed lung tissue occurs several weeks after radiotherapy. The mechanism for ACC may be that damage to the lung results in either, or both alveolar and interstitial edema, and that this stimulates a vagally mediated reflex which constricts the pulmonary vasculature. Cor pulmonale could thus result. Deep deliberate hypotension may be a result of the same reflex acting more slowly, and shallow deliberate hypotension may result from moderate lung edema retarding the flow of oxygenated blood to the heart.

A third mechanism by which vagal outflow can produce hypotension is by triggering extensive peripheral vasodilatation. Peripheral vasodilatation could be elicited by parasympathetic and sympathetic vagal activity. The ear temperature rise which accompanies ACC suggests that vasodilatation does occur after exposure. And, the ability of phenoxybenzamine, an alpha adrenergic blocker, to change the temperature response indicates that the temperature jump is neurally controlled. But, even though part of the adrenergic system was blocked with phenoxybenzamine, the acute fall in arterial pressure was not affected. This means that vasodilatation triggered by alpha adrenergic outflow is not critical to radiation hypotension. It may be that other components of the autonomic nervous system stimulate widespread vasodilatation, leading to a drop in blood pressure after radiation exposure. General neural outflow does seem to take place during acute hypotension, as evidenced by the presence of pupillary constriction, bladder emptying, and the loss of voluntary muscle tone. Furthermore, doubly vagotomized rabbits which are either whole body or heart irradiated, occasionally exhibit a sudden rise in blood pressure, which is accompanied by labored breathing and a sharp rise in ear temperature. It may be that this pressure rise was sympathetic in origin, and was unmasked because of the inactivation, by transection, of both vagi. Interestingly, histamine injected into the brain ventricles of anesthetized cats has been shown to cause a rise in blood pressure, presumably through stimulation of the sympathetic centers (Trendelenburg, 1957). The blood pressure records obtained after TBI or heart irradiation of doubly vagotomized rabbits resemble the pressure tracings for rabbits injected with endotoxin. Fukuda found that antipyretics could dimish the toxic symptoms of endotoxin in rabbits (Fukuda, 1963). He concluded that "endotoxin intoxication" in rabbits is due to central autonomic disturbances. It has been reported that endotoxin damage is mediated by the sympathetic nervous system (Thomas, 1956; Palmerio et al, 1962). Palmerio et al (1962) have demonstrated that following endotoxin injection in rabbits the right ventricle becomes dilated and inflamed. The lungs of rabbits are particularly susceptible to endotoxin; vasospasm of the lung vasculature is induced (Lillehei et al, 1964). It is possible that the neural outflow which leads to various manifestations after irradiation, is somehow related to the right ventricular pressure rise; ie, general autonomic outflow is accompanied by parasympathetic and/or sympathetic outflow which perhaps stimulates pulmonary vasoconstriction.

The main findings and essential interpretive points of this project are briefly recapitulated below.

1. The cardiovascular response of the rabbit to whole body irradiation has been characterized to produce a model for radiation hypotension of humans. ACC is a striking event, which is sensitive to experimental intervention, and has been shown to occur primarily in immature rabbits.

2. During acute hypotension the ECG often exhibits T-wave elevation, which is indicative of myocardial stress. This finding prompted selective irradiation/shielding of the heart region; this site was found to be an important target, which when selectively exposed, generated substantial deliberate pressure declines, and even acute hypotension. Irradiation of various structures in the zone of exposure, viz., the heart, lungs, neural structures, and blood, does not by itself cause deep hypotension. This conclusion stems from the results of heart shielding with double vagotomy; the blood and part of the lungs are exposed during heart shielding, but the hypotension generally tends to be relatively shallow. Also, selective irradiation of the heart with bilateral vagotomy results in shallow hypotension, which indicates that if it occurs, radiation damage to the heart is not responsible by itself for deep hypotension in intact animals subjected to TBI

3. Systemic histamine or other vasoactive compounds are not critical in radiation hypotension by triggering peripheral vasodilatation. Otherwise, heart shielding combined with double vagotomy would have little effect. Histamine may however, be very important in this syndrome by affecting the central nervous system. This question is highlighted by the use of antihistamines in irradiated rhesus monkeys; antihistamines and adrenalin partly maintained the blood pressure, but only antihistamines improved mental performance.

4. The results of single and double vagotomy, atropine, and

vagal monitoring all point to the likelihood that vagal transmission may be important in the hypotensive syndrome. The vagal monitoring has shown increased bulk vagal traffic after irradiation. Vagal cooling should be attempted again, since this technique would allow the complications of double vagotomy to be circumvented.

5. General neural efferent activity seems to arise in irradiated rabbits. The evidence of this is several-fold: the ear temperature rises sharply during acute hypotension; the pupil is often observed to constrict during ACC; the ear temperature jump takes place in doubly vagotomized rabbits; the post-exposure ear temperature profile is changed with sympathetic blockade; and, the blood pressure shows a characteristic, transient pressure rise in doubly vagotomized animals. The ear temperature rise suggests that peripheral vasodilatation may take place during ACC; this may be widespread throughout the body and it may be stimulated by neural efferents.

6. The right ventricular pressure recordings demonstrate that in acute, and in steep deliberate hypotension, the pressure in the right ventricle rises substantially, just before the onset of the arterial pressure decline. This pattern suggests that an obstruction to pulmonary flow may develop. Such an event can cause right heart failure and subsequent arterial hypotension. Further work on this hypothesis should involve isotopic measurements of lung blood perfusion and right ventricular pressure, in rabbits which

have both vagi cooled at selected times after exposure.

7. No specific practical therapy has been advanced by this work, except that it would appear that repeated doses of atropine may be worth trying in a critically ill radiation patient; this drug markedly reduced the incidence of acute hypotension, and marginally lessened the depth of the pressure decline. Also, neural disturbances in animals after fairly low doses have been demonstrated by various workers, and this present study has obtained indication of autonomic disturbances in exposed rabbits. Therefore, a combination of atropine with some sedative might be helpful: the sedative should have minimal blood pressure effects; perhaps mild anti-epilepsy drugs might be tried. Also, if pulmonary vascular constriction is important in radiation hypotension, perhaps the use of vasodilators which are specific to the lungs might be useful. In any case, the demonstration of heart region sensitivity, the results of vagotomy and vagal monitoring, and the the finding that the right ventricular pressure rises just prior to arterial hypotension, provide future research with a particular orientation with which to develop specific therapies. The present study revealed that dexamethasone, a corticosteroid, given as a single dose before TBI, was not at all effective. It may be that corticosteroids must be given over a relatively long period of time prior to exposure; this would render them relatively unattractive for treating immediate hypotension which might develop in radiation accident victims.

In conclusion, it should be stated that a definitive answer for radiation hypotension was not attained during this project. But, a concrete, practical experimental model was produced, the blood pressure decline was much reduced in heart shielded - doubly vagotomized rabbits, and strong circumstantial evidence has been adduced for a possible cor pulmonale-type mechanism which was heretofore not even postulated in relation to radiation hypotension. These steps extend beyond the body of knowledge presented in the Introduction, and raise new questions about a problem which has remained intractable for the past 63 years.

Tables

Table I: The Acute Response to TBI for 40-100 Day Old Rabbits

	Cannula	Cuff	Level of Significance
Pre-irradiation:			
Systolic	124.5 2.15 (45)	107.6 2.5 (29)	0.05
Diastolic	76.7 1.89 (45)		
Post-irradiation:			
Onset time (Min) of:			
Acute fall	64.5 1.8 (73)	71.1 2.31 (29)	0.05
Ear Temperature Rise	69.5 4.9 (13)†	67.1 2.7 (17)	--
Ear Temperature Rise °C	6.78 0.88 (14)	4.04 0.53 (17)	0.01
Time of Lowest Pressure (Min)	69.8 1.7 (72)		
Lowest Systolic	45.9 1.6 (72)		
Lowest Diastolic	28.2 1.0 (72)		
Decline from Pre-irradiation in Percent (of Mean Pressure)	61.8 1.4 (42)		
Final Time (Min)	96 3.4 (74)	79.9 2.8 (22)	0.025
Final Systolic	66.5 2.2 (72)	74.5 6 (6)	--
Final Diastolic	44.3 1.7 (72)		

†Onset time of Acute Fall for the 13 rabbits which had ear temperature measured and which showed the temperature rise was 69.9 min.(SE=4.74)

*SE's are given, numbers of rabbits are in brackets.

*Levels at which cannula and cuff measurements differ significantly are given (Student's t-test). A bar indicates very little or no significant difference.

*Cannula doses 1020-1260 cGy; cuff doses 978-4587 cGy.

Table II: Hematocrit Values for Cannulated and Nonoperated Rabbits

Rabbit I.D.	Cannulated or Nonoperated	Irradiated or Sham	Hematocrits Before Irradiation % Red Cells			Hematocrits After Irradiation % Red Cells						Time (min.) Samples Taken Before Irradiation and How	Time (min.) Samples Taken After Irradiation and How
			1	2	3	1	2	3	Drop 1	2	3		
2NN1	nonop	1200 cGy	40.8	-	-	38.4	-	-	-	-	-	33 ear artery	101 cardiac puncture
2NN3	nonop	1200 cGy	38.4	-	-	42	-	-	-	-	-	28 ear artery	67 ear artery
2NN2	nonop	1200 cGy	43.2	-	-	40.8	-	-	-	-	-	27 ear artery	66 ear artery
2NR1	nonop	1200 cGy	40.8	-	-	35.4	-	-	-	-	-	21 ear artery	101 cardiac puncture
2NO8	nonop	1200 cGy	42.6	-	-	42	-	-	-	-	-	19 ear artery	64 ear artery
2NP1	nonop	1200 cGy	38.4	-	-	36	-	-	-	-	-	17 ear artery	92 cardiac puncture
* 2NR3	nonop	Sham	40.8	39.6	-	40.2	39.6	39.6	-	-	-	25 ear artery	57 ear artery
* 2NO2	nonop	Sham	43.2	42	43.2	44.4	43.2	45	-	-	-	19 ear artery	59 ear artery
* 2Z3	nonop	Sham	40.8	42.6	45	45	45.6	45	-	-	-	13 ear artery	61 ear artery
2NO5	cannulated A	1200 cGy	45.6	-	-	49.2	-	-	42.6	-	-	5 dorsal aorta	9, 68 dorsal aorta
2NO1	cannulated A	1200 cGy	46.8	-	-	46.8	-	-	44.4	-	-	12.5 dorsal aorta	10, 61.5 dorsal aorta
* 2NQ1	cannulated A	1200 cGy	44.4	43.2	45.6	45.6	44.4	45.6	40.2	42.6	40.2	35-40 dorsal aorta	7, 83 dorsal aorta
* 2NQ2	cannulated	Sham	44.4	36	44.4	45.6	45.6	46.8	42	42.6	42	16 dorsal aorta	5-11, 64 dorsal aorta

* Means samples determined in triplicate.

"A" denotes acute responder

Table III: The Deliberate Response to TBI for 40-100 Day Old Rabbits

	Cannula	Cuff	P
Pre-irradiation:			
Systolic	126.5 4.7 (15)	102.2 3.4 (27)	0.01
Diastolic	75.3 2.9 (23)		
Post-irradiation:			
Time of lowest Pressure (Min)	95.9 5.46 (24)	90.7 7 (22)	--
Lowest Systolic	77.6 4.87 (24)	67.9 3.1 (22)	0.2
Lowest Diastolic	48.3 2.86 (24)		
Decline from Pre-irradiation in Percent (of Mean Pressure)	34.7 5.27 (13)		
Decline from Pre-irradiation in Percent (of Systolic Pressure)	33.3 4.98 (13)	34.5 2.83 (23)	--
Time of Final Pressure (Min)	125 6.4 (25)	118.2 4.3 (20)	--
Final Systolic	83.4 4.74 (25)	85.4 5.9 (19)	--
Final Diastolic	56.4 2.94 (25)		

* SE's are given, numbers of rabbits are in brackets.

* Levels at which cannula and cuff measurements differ significantly are given (student's t-test). A bar indicates very little or no significant difference.

* Cannula doses 1092-1206 cGy; cuff doses 978-4587 cGy.

Table IV: Survival of Rabbits Following Various Procedures

Procedure	Number of Animals	Age Range (Days)	Irradiation Dose/Doserate (cGy @ cGy/min.)	Average Survival (Days)	Survival Range (Days)	Significant Difference vs. 40 - 100 Day Cannulated Exposed to 1260 - 1071.6 cGy**
Not starved no anesthesia	4	55-73	1200 @ 200 or 1105.2 - 1092 @ 1473.6 - 1456	8.1	7 - 8.7	yes, α = 0.010
Starved, anesthesia	5*	56-65	1102.8 @ 1470 or 1093.2 @ 1457.6	5.9	0.6 - 9.7	yes, α = 0.010
Sham - femoral artery tied off	5	51-61	1102.8 - 1071.6 @ 1470.4 - 1428.8 2191.2 @ 1460.8	6.9	4.6 - 9.7	yes, α = 0.010
	1	62		5.8	5.8	
Femoral artery Cannulated	48	43-98	1260 - 1071.6 @ 1680 - 1428.8	1.57	0.08 - 8.0	
Femoral artery Cannulated	5	158-373	1200 @ 200	0.54	0.1 - 0.8	
Femoral artery Cannulated	5	65-84	5088 @ 1696	0.94	0.6 - 1.3	
Femoral artery Cannulated	5	65-68	2522.4 or 2520 @ 1681.6, 1680	0.59	0.07 - 1	
Femoral artery Cannulated	2	63	628.8 @ 1676.8	1.45	1.2, 1.7	
Femoral artery Cannulated	2	128,161	NONE	1, 4	1, 4	
Femoral artery	2	57	NONE	Terminated at 2 and 4 days respectively due to complications caused by cannulation	2, 4	

* A 58 day old rabbit in this group survived only 0.6 days, well outside the range for the other four animals, of 6 - 9.7 days. Could not be rejected by Q-test. Q 90% value = 0.64, calculated Q = 0.59

** Student's t-test.

Table V: Incidence of Acute Hypotension According to Dose for Cannula and Cuff Monitored Rabbits

Dose Range (Gy)	Cannula	Cuff	Total
6 - 9.5	3/5 = 60%	-	3/5 = 60%
9.5 - 15.5	75/103 = 72.8%	11/22 = 50%	86/125 = 68.8%
15.5 - 28.5	4/5 = 80%	15/25 = 60%	19/30 = 63.3%
28.5 - 50.9	4/5 = 80%	3/10 = 30%	7/15 = 46.7%

Table VI: Acute Hypotension in Three Groups of 40 - 100 Day Rabbits Irradiated With Three Different Radiation Sources

Mean Blood Pressure (mm Hg)	6 MeV X-ray	Gammacell 220 (co-60)	Theratron 80/780 (co-60)
Before Irradiation	93.1 2.33 (13)	95.3 3.15 (20)	87.2 2.14 (12)
Minimum After Irradiation	32 1.68 (22)	34.6 1.76 (38)	36.1 2.18 (12)
Percent Decline	62.4 2.12 (13)	63.7 2.49 (17)	58.5 2.5 (12)
Time of Onset, Acute Fall	58 2.72 (22)	64.9 2.1 (40)	76.1 6.2 (11)
Incidence Acute Fall	70.1% (22/31)	74.5% (41/55)	75% (12/16)

Note: * Numbers of animals are in brackets.

* Time of acute onset differs at 5% level between 6MeV x-ray and Gammacell 220 groups (Student's t-test).

* Time of acute onset differs at 2.5% level between Gammacell and Theratron groups (Student's t-test).

* Fisher's exact probability shows no difference between the 6 MeV x-ray group and the Gammacell group in terms of the incidence of the acute fall ($P<0.2$).

* All animals 40-100 days old and cannulated.

* Dose rates with 6 MeV X-ray 91.2 and 200 cGy/minute; with Gammacell 892.8-1680 cGy/minute; and with Theratron 91-110 cGy/minute.

Table VII: Degree of Power Spectrum Nerve Profile Definition at Various Times After Cuff Implantation

Grading Scheme:

E - Excellent bipartite profile
VG - Very good
G - Good
F - Fair
P - Poor

Rabbit	Days After Implantation				
	0	1	2	3	4
1	VG	E	-	-	P
2	-	P	-	-	-
3	VG	G	-	-	-
4	P	P	-	-	-
5	F	VG	-	-	P
6	P	P	-	-	-

Table VIII: Blood Pressure in Inferior Vena Cava After co-60 Whole Body Irradiation

Pressure in mm Hg.

	Dorsal Aorta (mean pressure)	Inferior Vena Cava (Systolic/Diastolic)	Average IVC
Rabbit 1			
Before Irradiation			
Lowest	83.5		
Average over time	85.2		
Highest	89.5	9.6/ 4.7	6.3
After Irradiation			
10 minutes	94.2	0.7/<0	0.23
30 minutes	91.8	7.4/ 1.1	3.2
60 minutes	79.4	1	
Onset of aortic decline (@ 62 mins)	78.4	6.7/<0	2.2
Onset of IVC rise (@ 78.6 mins)	36.6	8.5/<0	2.8
End of aortic decline (@ 79.4 mins)	28.9	9.8/<0	3.2
Peak of IVC pressure (@ 95.2 mins)	31	17.8/ 8.5	11.6
Final (@ 106.7 mins)	34.1	15.8/ 7.3	10.1
Rabbit 2			
Before Irradiation			
Highest	-	26.3/<0	8.7
After Irradiation			
10 minutes	-	29 /<0	9.6
30 minutes	-	28.1/<0	9.3
75 minutes	-	21.5/ 4	9.8
Onset of IVC rise (@ 78.6 mins)	-	22.5/ 3.4	9.7
Peak IVC pressure (@ 89.6 mins)	-	33.8/10.5	18.2
Final (@ 95.8 mins)	-	28.5/10.5	16.4

Table IX: Right Ventricular Measurement in Whole Body Irradiated Rabbits

Pressure (mm Hg)	Dorsal Aorta (lowest pressure)			Right Ventricle (highest pressure)		
Pre-irradiation:						
Systolic	106.8	4.8	(9)	37.4	2.6	(9)
Diastolic	56.3	2.9	(9)	0.28	0.28	(9)
Mean	72.9	3.4	(9)	12.5	0.88	(9)
Post-irradiation:						
Systolic	56.6	3.8	(8)	52.6	2.8	(9)
Diastolic	31.8	1.9	(8)	0.11	0.11	(9)
Mean	40.0	2.5	(8)	17.4	0.96	(9)
Times of lowest/highest post-irradiation pressures (minutes)	92.4	6.4	(8)	61.4	7.6	(9)
Percent Decline/Rise:						
Systolic	48.2	2.8	(8)	47	13	(9)
(range)	(33.9 - 57.1)			(-11.1 - 108.7)		
Diastolic	45.4	1.5	(8)	6.7	6.7	(9)
(range)	(39.4 - 52)			(0 - 60)		
Mean	46.8	2	(8)	45.3	12.8	(9)
(range)	(36.9 - 52.6)			(-11.1 - 119.8)		

*Note: Standard errors are given, and numbers of animals are in brackets.

Table X: Percentage Drift for Various Blood Pressure Recording Systems

Recording System	% Drift for Zero Baseline (Range, Std. Dev.)	% Drift Full Scale Line (Range, Std. Dev.)
SMRI - Beckman chart recorder and Gould P23 ID transducer	1.15 (0 - 3.5) SD = 1.05, n = 10	1.15 (0 - 2.8) SD = 0.88, n = 7
Beckman R411 recorder and Gould P23 ID transducer (Before R411 servicing)	2.4 (0 - 10.1) SD = 3.15, n = 15	2.5 (0 - 12.1) SD = 3.19, n = 13
Beckman R411 recorder and Gould P23 ID transducer (After R411 servicing)	0.45 (0 - 2.5) SD = 0.56, n = 26	0.64 (0 - 5) SD = 1.11, n = 20
Gould 2400S recorder and Gould P23 ID transducer	0.7 (0 - 2) SD = 0.51, n = 63	0.13 (0 - 1.5) SD = 0.31, n = 62
Gould 2400S recorder and Millar catheter tip transducer for external use	1.7 (0 - 5.3) SD = 1.45, n = 10	2 (0.7 - 3.1) SD = 0.78, n = 9
Cuff Apparatus - Beckman R411 recorder and Beckman Indirect Blood Pressure Coupler	1.5 (0 - 3.8) SD = 0.85, n = 64	-- -- --

Table XI: Cuff and Cannula Derived Blood Pressures Taken Simultaneously in the Same Rabbits Before and at Various Times After Whole Body Irradiation

Pre-irradiation (All are averages of 6 readings, and ranges are in brackets)

	Cuff (systolic)	Cannula (systolic)	Cannula (diastolic)
Rabbit 1	116.1 (108-128)	118 (111-120)	--
Rabbit 2	99 (85-102)	141.3 (132-147.5)	78.5 (70-84)

Post-irradiation (Single readings)

Rabbit 1

Time After Irradiation Minutes	Cuff (systolic)	Cannula (systolic)	Cannula (diastolic)
23	82	148	85
43	93	135	77
55	86	126	74
63	66	120	70
65	55	91	53
67	Can't Read	40	20.5

Figure 1. Systolic and diastolic pressures for 29 patients plotted according to time after UHBI with 800 cGy. The systolic pressure and the diastolic pressure for each time represents the average for all 29 individuals together, and is expressed as the percentage of the systolic and diastolic pressure respectively, which were taken immediately after irradiation. The figure was constructed using clinical data which were kindly provided by Dr. R. C. Urtasun of the W. W. Cross Cancer Institute, in Edmonton, Canada.

Fig. 1 Effect of UHBI on Blood Pressure

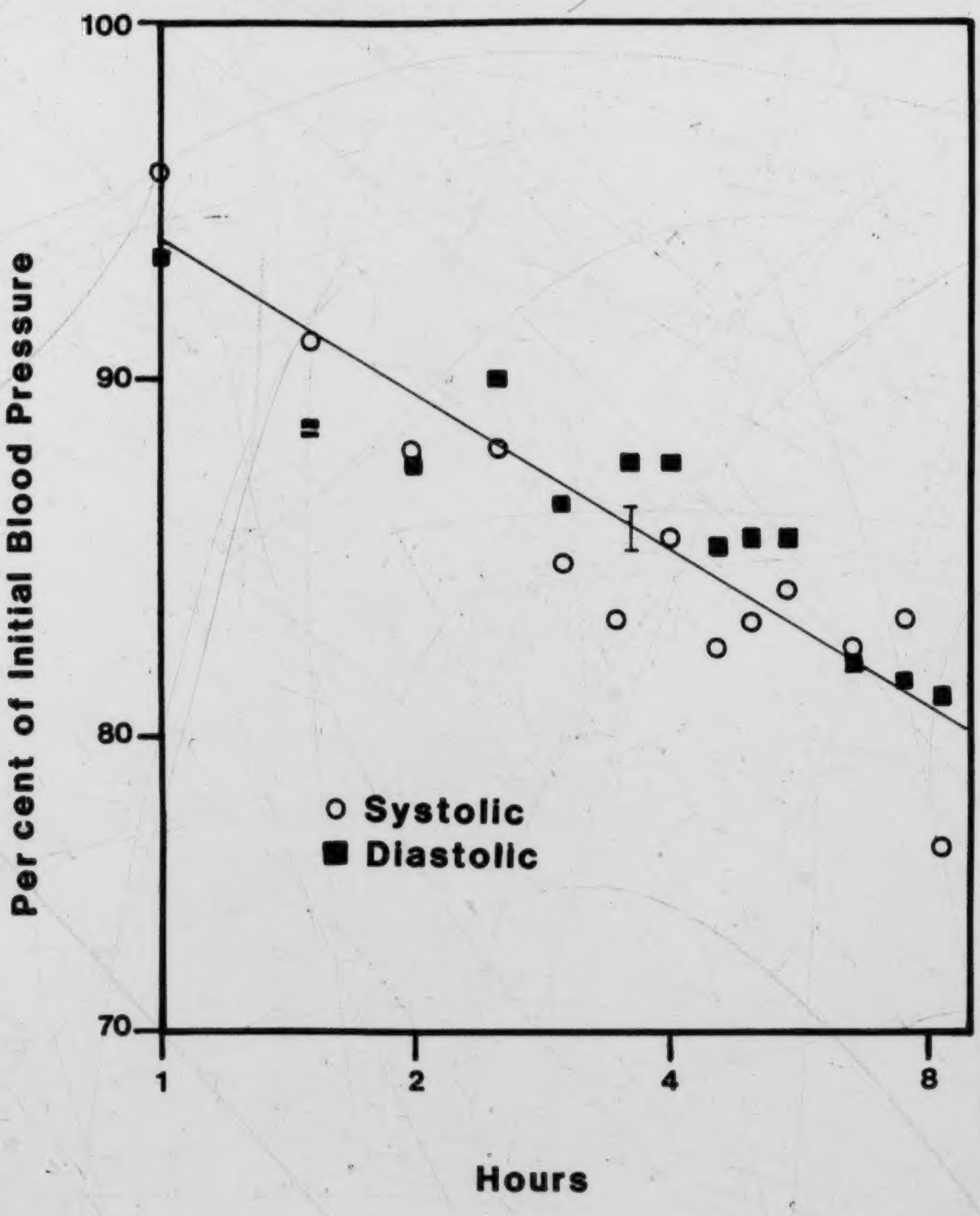


Figure 2. Apparatus for direct measurement of aortic and right ventricular blood pressure. The diagram shows the Gould P23 ID external transducer which was used for aortic and right ventricular pressure measurement. Also depicted are the Millar catheter transducer and the Millar control unit. The diagram shows the Millar system arranged for calibration just prior to use externally for the measurement of aortic pressure in rabbits which were catheterized in the right ventricle.

FIGURE 2

DIRECT PRESSURE MEASUREMENT APPARATUS

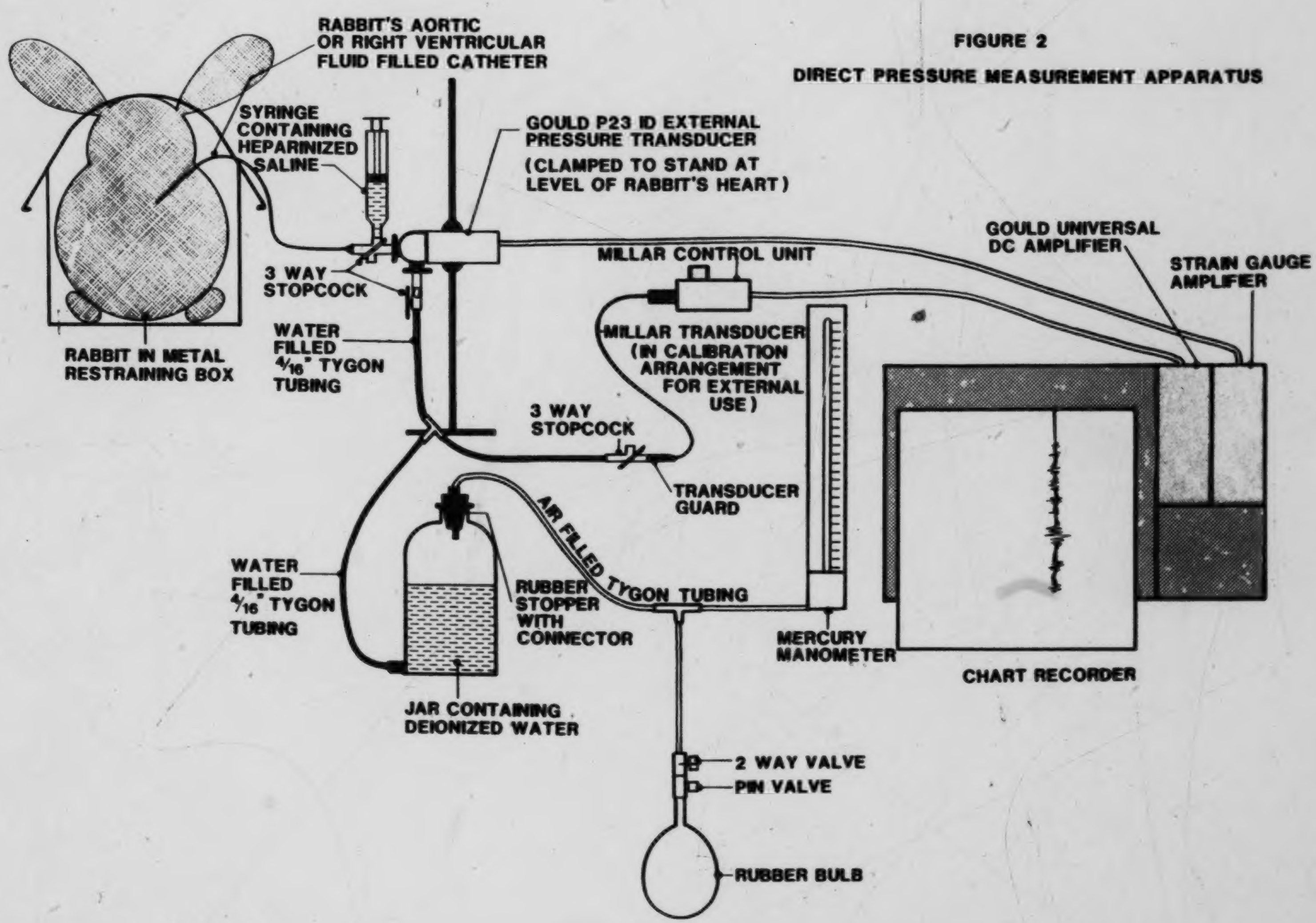

RABBIT'S AORTIC OR RIGHT VENTRICULAR FLUID FILLED CATHETER
SYRINGE CONTAINING HEPARINIZED SALINE
GOULD P23 ID EXTERNAL PRESSURE TRANSDUCER
(CLAMPED TO STAND AT LEVEL OF RABBIT'S HEART)
3 WAY STOPCOCK
RABBIT IN METAL RESTRAINING BOX
WATER FILLED 4/16" TYGON TUBING
MILLAR CONTROL UNIT
GOULD UNIVERSAL DC AMPLIFIER
STRAIN GAUGE AMPLIFIER
MILLAR TRANSDUCER (IN CALIBRATION ARRANGEMENT FOR EXTERNAL USE)
3 WAY STOPCOCK
TRANSDUCER GUARD
AIR FILLED TYGON TUBING
WATER FILLED 4/16" TYGON TUBING
RUBBER STOPPER WITH CONNECTOR
JAR CONTAINING DEIONIZED WATER
MERCURY MANOMETER
CHART RECORDER
2 WAY VALVE
PIN VALVE
RUBBER BULB

Figure 3. Pressure waveform apparatus for dynamic testing of frequency response. The bellows was directed to produce a pressure square wave by the movement of the loudspeaker cone. The electrical square wave signal was supplied by the Hewlett Packard 3310A function generator.

FIGURE 3

PRESSURE WAVE APPARATUS

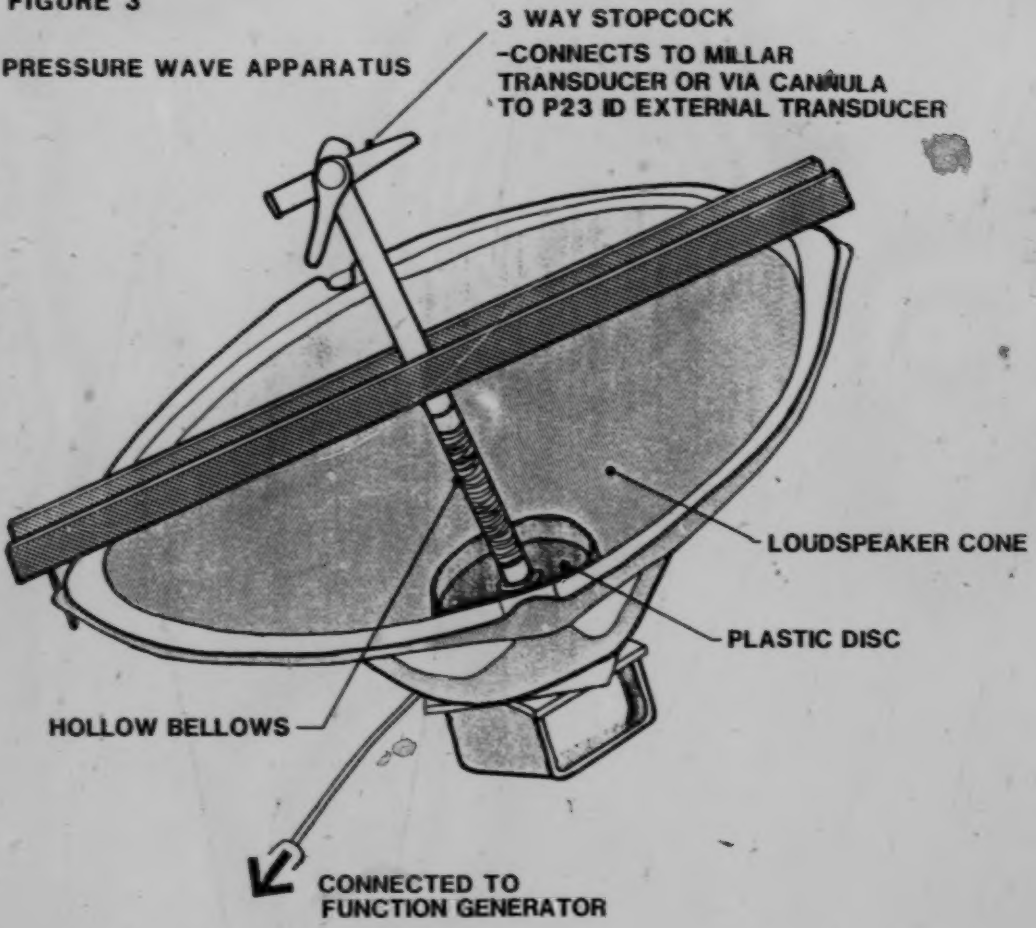

3 WAY STOPCOCK
-CONNECTS TO MILLAR TRANSDUCER OR VIA CANNULA TO P23 ID EXTERNAL TRANSDUCER
LOUDSPEAKER CONE
PLASTIC DISC
HOLLOW BELLOWS
CONNECTED TO FUNCTION GENERATOR

Figure 4. Tail cuff apparatus for indirect measurement of systolic arterial blood pressure. When the cuff was pressurized to 200 mm Hg, blood flow into the tail was blocked, and the pulse transducer was inactive. However, when the pressure within the cuff declined to approximate equivalence with the systolic arterial pressure, blood flow resumed and the transducer generated a pulse signal. The transducer and cuff were wrapped to the tail with an elastic adhesive bandage. Thus, both attachments moved with the tail, thereby minimizing mechanically generated noise.

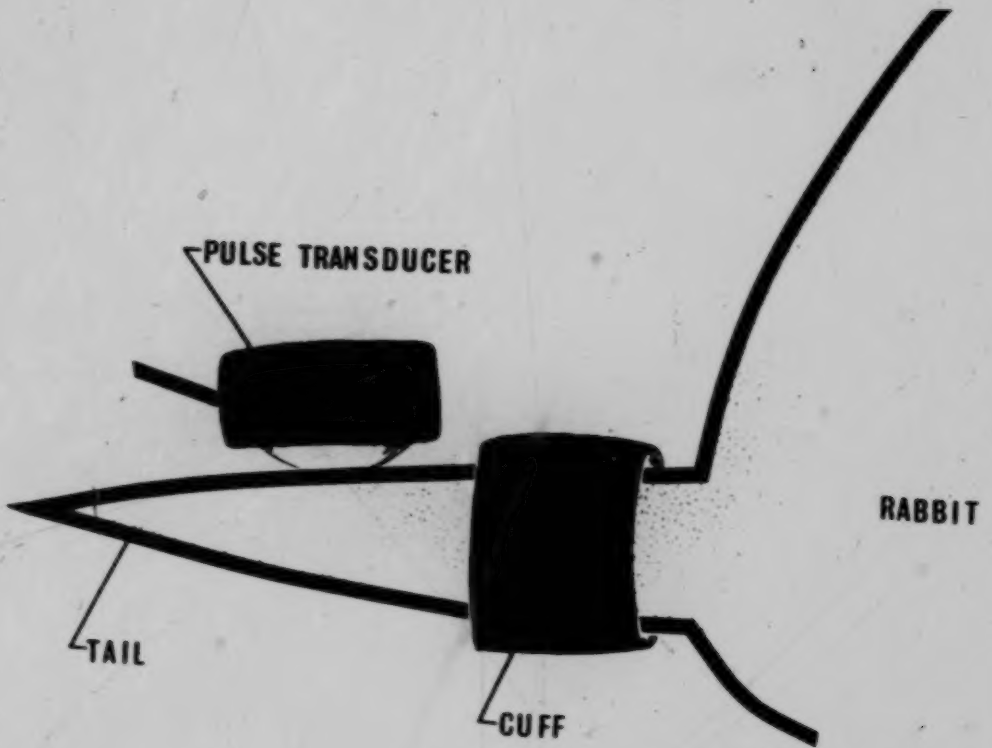

PULSE TRANSDUCER
RABBIT
TAIL
CUFF

Figure 4. Tail cuff technique of blood pressure measurement.

Figure 5. Control and recording apparatus for the indirect measurement of systolic arterial pressure. The 9863A indirect blood pressure coupler combined the air pressure and pulse signals to produce the tracing represented by Plate 3.

FIGURE 5 TAIL CUFF CONTROL AND RECORDING APPARATUS

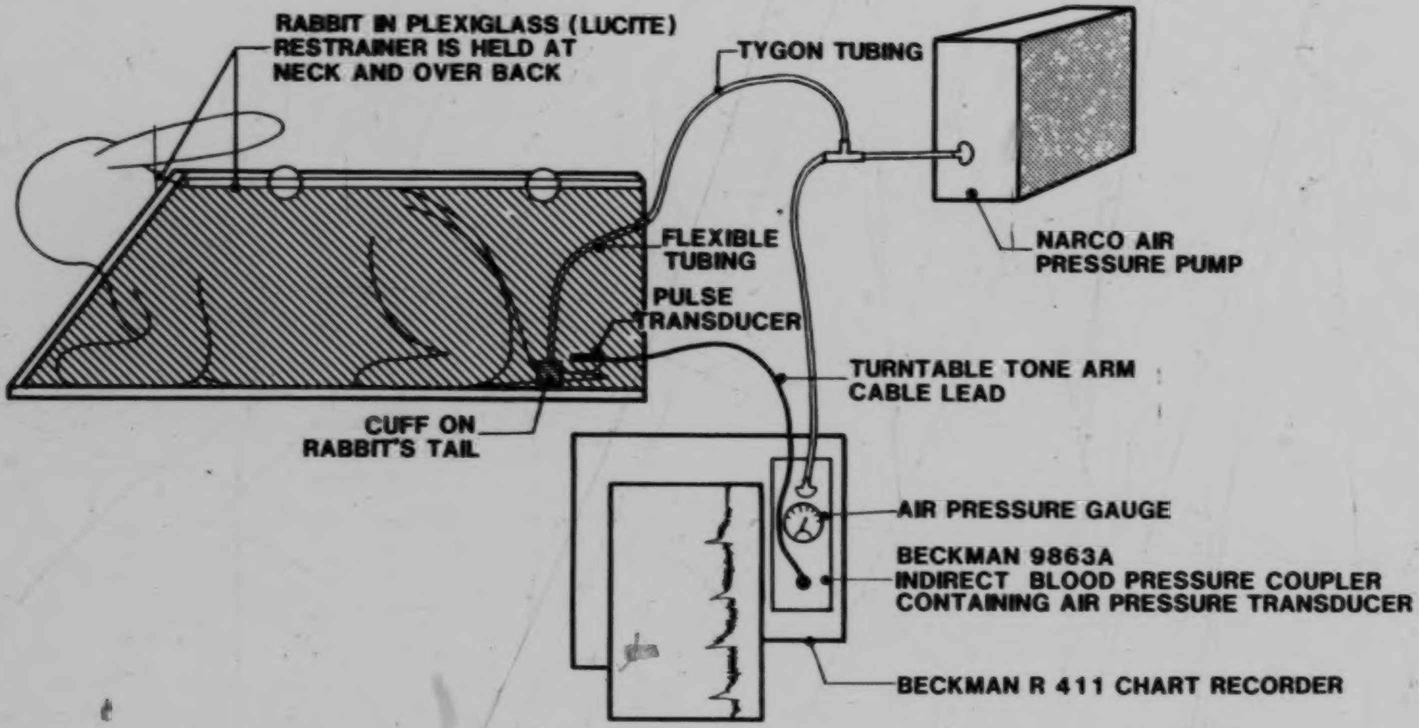
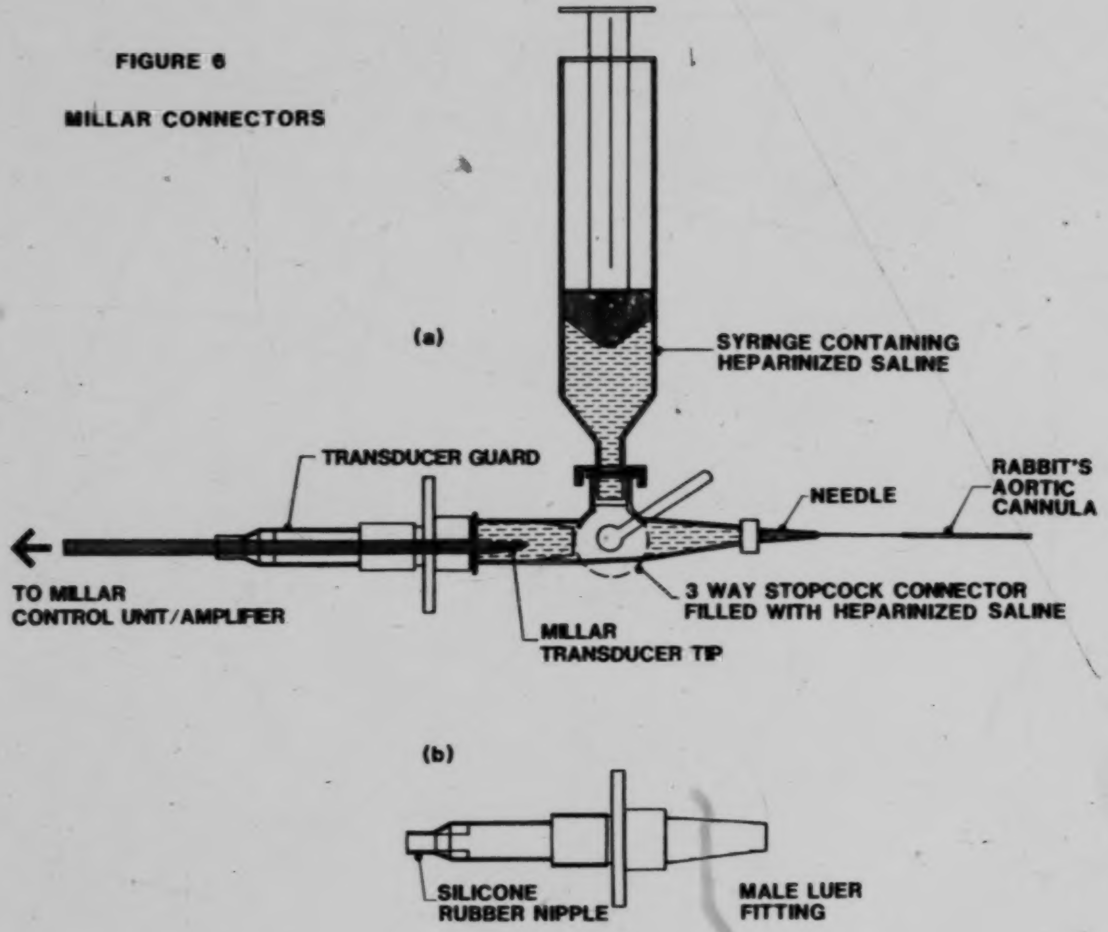

RABBIT IN PLEXIGLASS (LUCITE) RESTRAINER IS HELD AT NECK AND OVER BACK
TYGON TUBING
FLEXIBLE TUBING
PULSE TRANSDUCER
NARCO AIR PRESSURE PUMP
TURNTABLE TONE ARM CABLE LEAD
CUFF ON RABBIT'S TAIL
AIR PRESSURE GAUGE
BECKMAN 9863A INDIRECT BLOOD PRESSURE COUPLER CONTAINING AIR PRESSURE TRANSDUCER
BECKMAN R 411 CHART RECORDER

Figure 6. (a) Method of connecting the Millar catheter transducer to the rabbit's aortic cannula for measurement of aortic pressure.

(b) Transducer guard; also shown in use by Figure 6 (a).

FIGURE 6

MILLAR CONNECTORS


(a)
SYRINGE CONTAINING HEPARINIZED SALINE
TRANSDUCER GUARD
NEEDLE
RABBIT'S AORTIC CANNULA
TO MILLAR CONTROL UNIT/AMPLIFIER
3 WAY STOPCOCK CONNECTOR FILLED WITH HEPARINIZED SALINE
MILLAR TRANSDUCER TIP
(b)
SILICONE RUBBER NIPPLE
MALE LUER FITTING

TRANSDUCER GUARD

Figure 7. Block diagram showing the equipment used, and the flow of data, in computer directed physiological monitoring. The chart is divided into two broad categories: (1) the measurement of various physiological parameters and the processing of data, and (2) the storage of data.

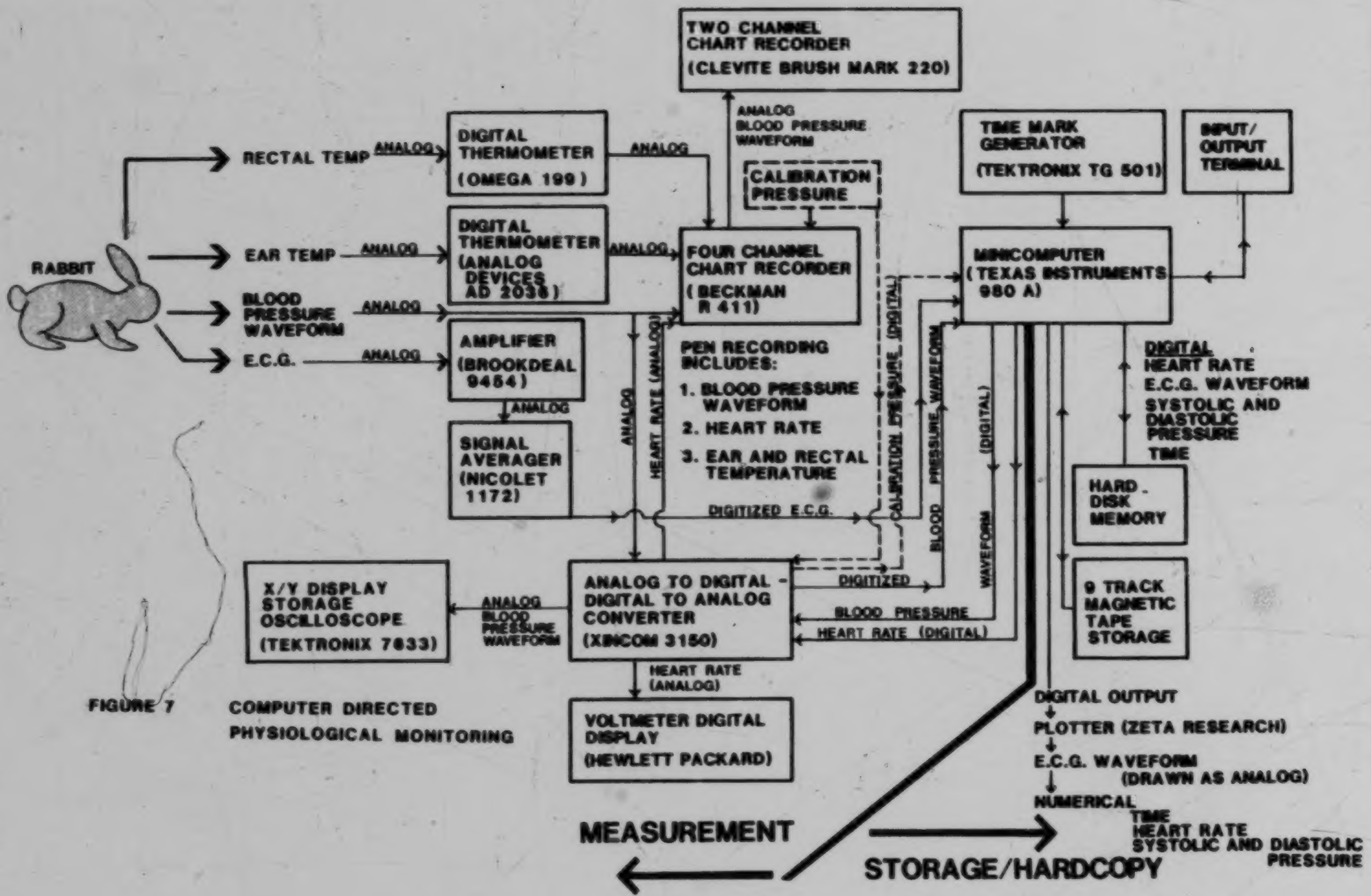

RABBIT
RECTAL TEMP
EAR TEMP
BLOOD PRESSURE WAVEFORM
E.C.G.
ANALOG
DIGITAL THERMOMETER (OMEGA 199)
DIGITAL THERMOMETER (ANALOG DEVICES AD 2036)
AMPLIFIER (BROOKDEAL 9454)
SIGNAL AVERAGER (NICOLET 1172)
TWO CHANNEL CHART RECORDER (CLEVITE BRUSH MARK 220)
ANALOG BLOOD PRESSURE WAVEFORM
CALIBRATION PRESSURE
FOUR CHANNEL CHART RECORDER (BECKMAN R 411)
PEN RECORDING INCLUDES:
1. BLOOD PRESSURE WAVEFORM
2. HEART RATE
3. EAR AND RECTAL TEMPERATURE
HEART RATE (ANALOG)
DIGITIZED E.C.G.
CALIBRATION PRESSURE (DIGITAL)
BLOOD PRESSURE WAVEFORM
WAVEFORM (DIGITAL)
DIGITIZED
BLOOD PRESSURE
HEART RATE (DIGITAL)
TIME MARK GENERATOR (TEKTRONIX TG 501)
INPUT/ OUTPUT TERMINAL
MINICOMPUTER (TEXAS INSTRUMENTS 980 A)
DIGITAL
HEART RATE
E.C.G. WAVEFORM
SYSTOLIC AND DIASTOLIC PRESSURE
TIME
HARD DISK MEMORY
9 TRACK MAGNETIC TAPE STORAGE
X/Y DISPLAY STORAGE OSCILLOSCOPE (TEKTRONIX 7633)
ANALOG BLOOD PRESSURE WAVEFORM
ANALOG TO DIGITAL - DIGITAL TO ANALOG CONVERTER (XINCOM 3150)
HEART RATE (ANALOG)
VOLTMETER DIGITAL DISPLAY (HEWLETT PACKARD)
DIGITAL OUTPUT
PLOTTER (ZETA RESEARCH)
E.C.G. WAVEFORM (DRAWN AS ANALOG)
NUMERICAL
TIME
HEART RATE
SYSTOLIC AND DIASTOLIC PRESSURE
MEASUREMENT
STORAGE/HARDCOPY

FIGURE 7 COMPUTER DIRECTED PHYSIOLOGICAL MONITORING

Figure 8. Block diagram showing equipment used in power spectrum monitoring of vagal transmission. The point of entry of the calibration signal is also shown.

FIGURE 8

POWER SPECTRUM APPARATUS

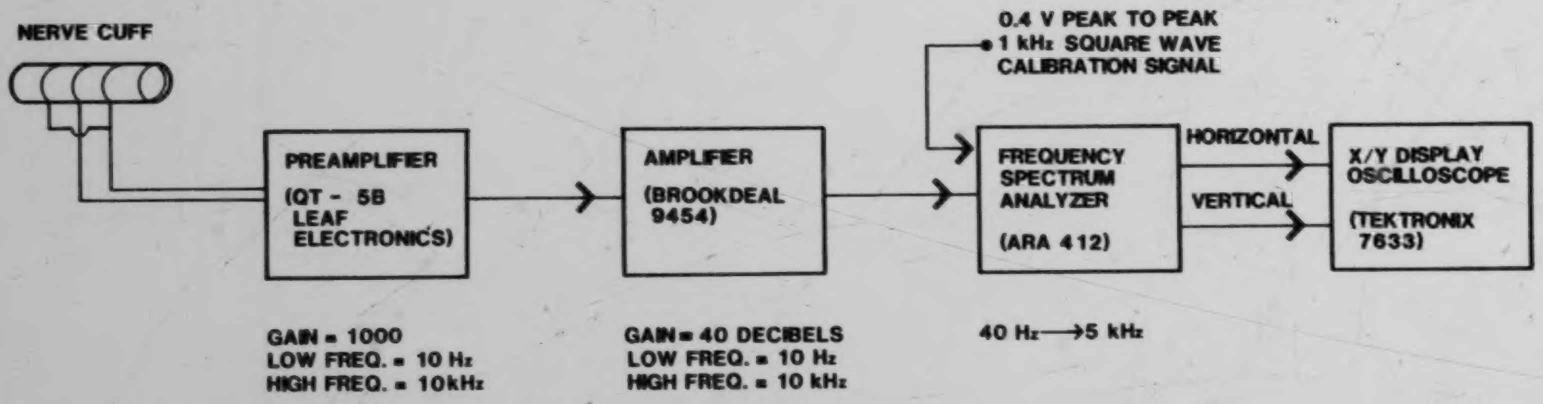

NERVE CUFF
PREAMPLIFIER
(QT - 5B
LEAF
ELECTRONICS)
AMPLIFIER
(BROOKDEAL
9454)
0.4 V PEAK TO PEAK
1 kHz SQUARE WAVE
CALIBRATION SIGNAL
FREQUENCY
SPECTRUM
ANALYZER
(ARA 412)
HORIZONTAL
VERTICAL
X/Y DISPLAY
OSCILLOSCOPE
(TEKTRONIX
7633)
GAIN = 1000
LOW FREQ. = 10 Hz
HIGH FREQ. = 10kHz
GAIN = 40 DECIBELS
LOW FREQ. = 10 Hz
HIGH FREQ. = 10 kHz
40 Hz → 5 kHz

Figure 9. (a) Diagram of base plate and connectors for the vagus cooler. The figure is drawn with the covering plate removed.

(b) A diagram showing the longitudinal cross section of the vagus cooler tip cylinder. For both 9 (a) and (b) an impression of scale may be gained by referring to Plate 6.

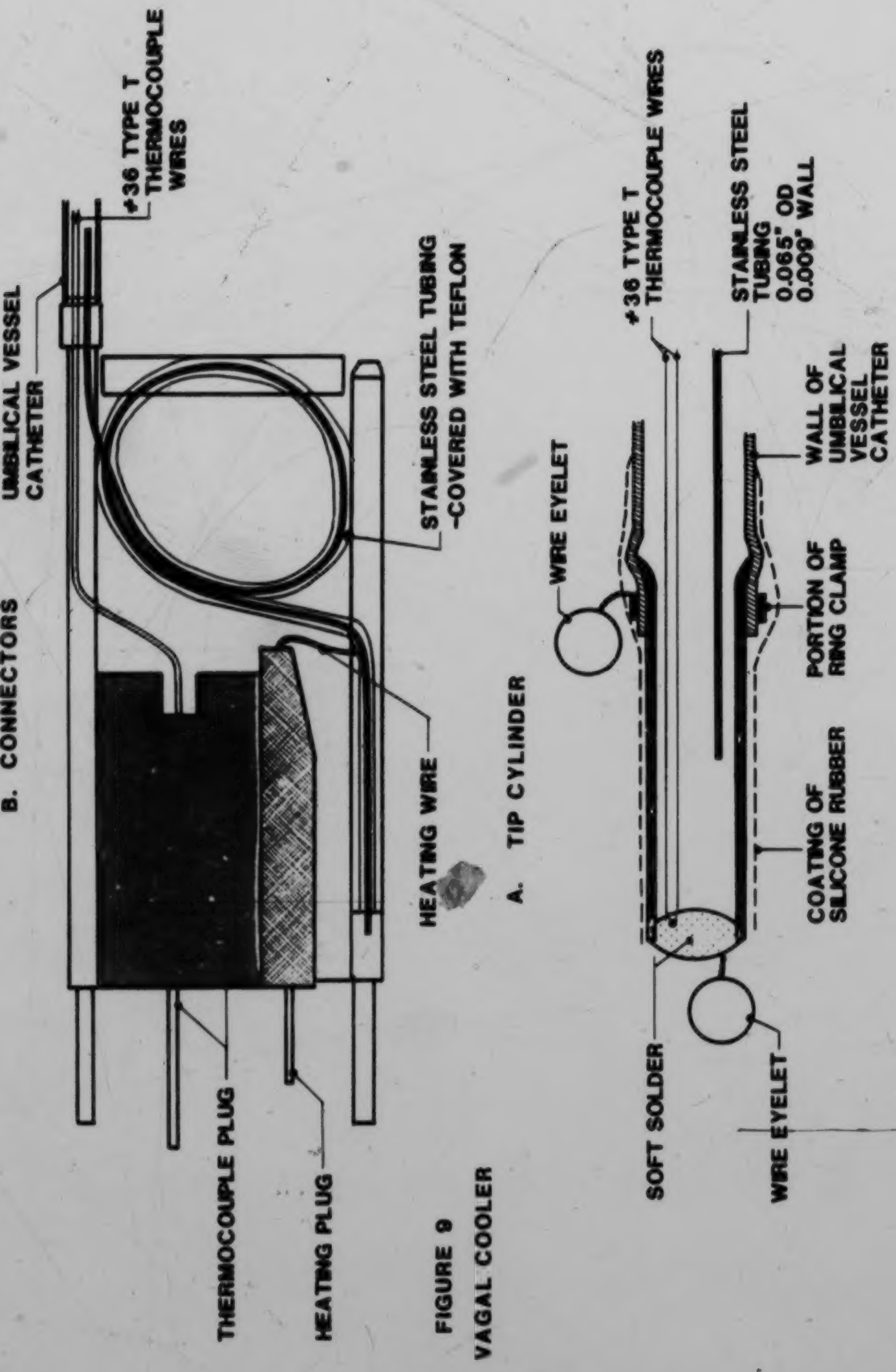


FIGURE 9
VAGAL COOLER

A. TIP CYLINDER

B. CONNECTORS

UMBILICAL VESSEL CATHETER
#36 TYPE T THERMOCOUPLE WIRES
STAINLESS STEEL TUBING -COVERED WITH TEFLON
HEATING WIRE
THERMOCOUPLE PLUG
HEATING PLUG
WIRE EYELET
#36 TYPE T THERMOCOUPLE WIRES
STAINLESS STEEL TUBING 0.065" OD 0.009" WALL
WALL OF UMBILICAL VESSEL CATHETER
PORTION OF RING CLAMP
COATING OF SILICONE RUBBER
SOFT SOLDER
WIRE EYELET

Figure 10. Time versus pressure chart containing a band which depicts the aortic blood pressure response of ten 40 to 100 day old rabbits to sham irradiation. The vertical scale is pressure in mm Hg, and the horizontal scale indicates the time in minutes after the beginning of sham irradiation. The hatched band was produced by first averaging the systolic and the diastolic pressures at various times after sham irradiation for the ten rabbits. The average systolic and the average diastolic values were plotted on the chart, and the systolic points were connected with a line, as were the diastolic points. The area between the systolic line and the diastolic line was filled in with hatching. The black circles represent the averaged lowest systolic and averaged lowest diastolic values for the ten rabbits, taken before sham irradiation. The pressure band shows a slight dip commencing at about 60 minutes after sham. The pressure band shows a slight dip commencing at about 60 minutes after sham. The reason for this is unknown, although it may have been due to the physical circumstances of restraint; perhaps the rabbit became accustomed to the restraining box, relaxed somewhat, and the blood pressure declined slightly. It is possible that cause of the pressure dip which occurred at 60 minutes in sham rabbits, also predisposed irradiated rabbits to experience ACC at about 60 minutes after exposure. In any event, the post-sham blood pressure did not fall below the lowest pre-sham pressure, ie., no net decline in mean arterial pressure occurred with the sham group. (Although individual animals did exhibit slight pressure reductions.)

Fig. 10: Response to SHAM Irradiation; 40–100 Days

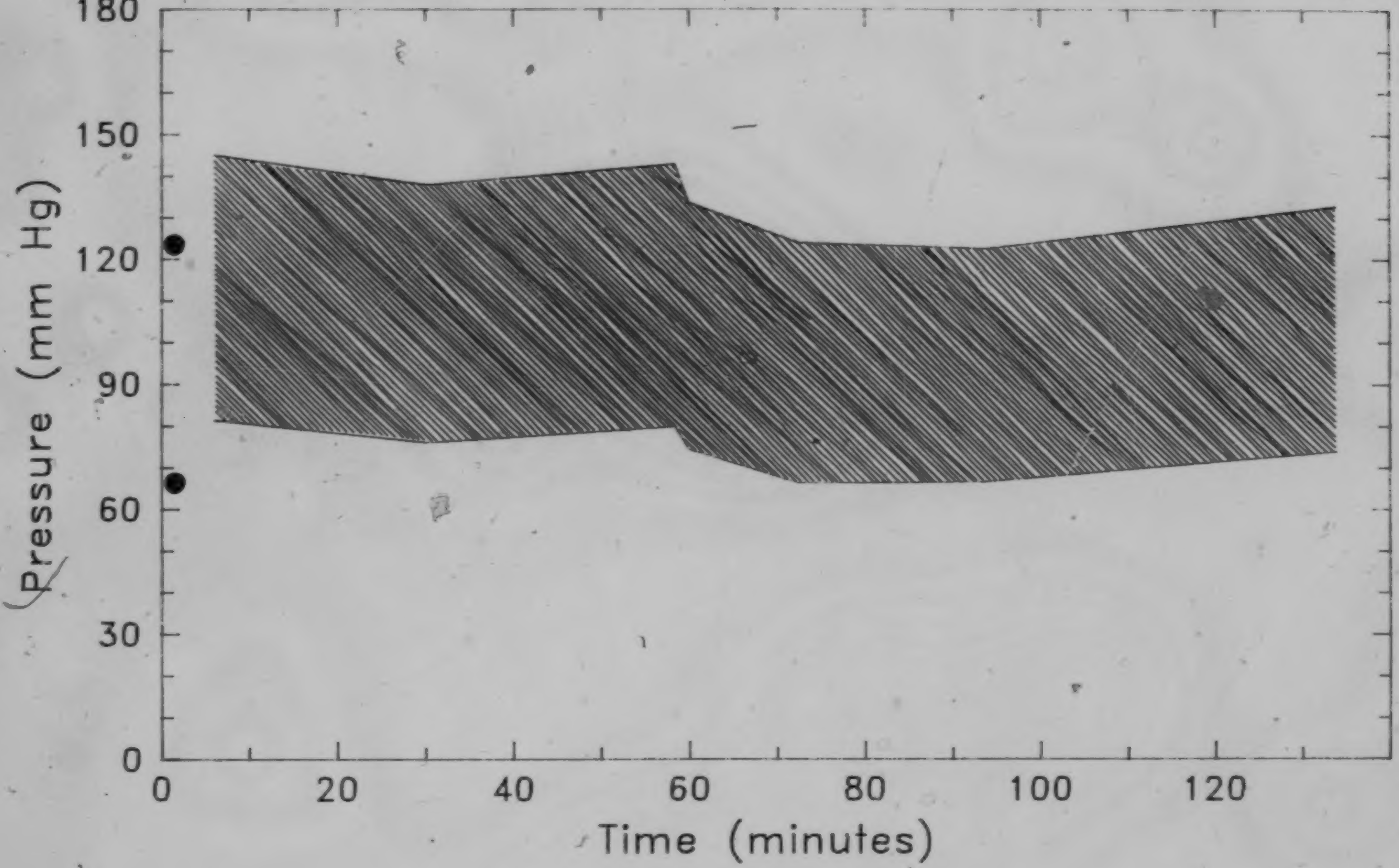


Figure 11. Reproduction of the chart recording obtained from a 55 day old rabbit which experienced ACC after TBI with 1190.4 cGy. The topmost tracing is of aortic blood pressure, and at 66 minutes following TBI the acute drop began. The vertical scale is from 0 to 200 mm Hg. The time scale for the entire chart recording is indicated in minutes from the beginning of TBI, just below the pressure trace. The second tracing from the top is of rectal temperature. The vertical scale from 51 minutes onward is from 38 to 40 degrees centigrade. The third tracing is the heart rate, and the vertical scale is from 0 to 500 BPM. The bottom tracing is of ear temperature, and the scale is from 24 to 40 degrees centigrade.

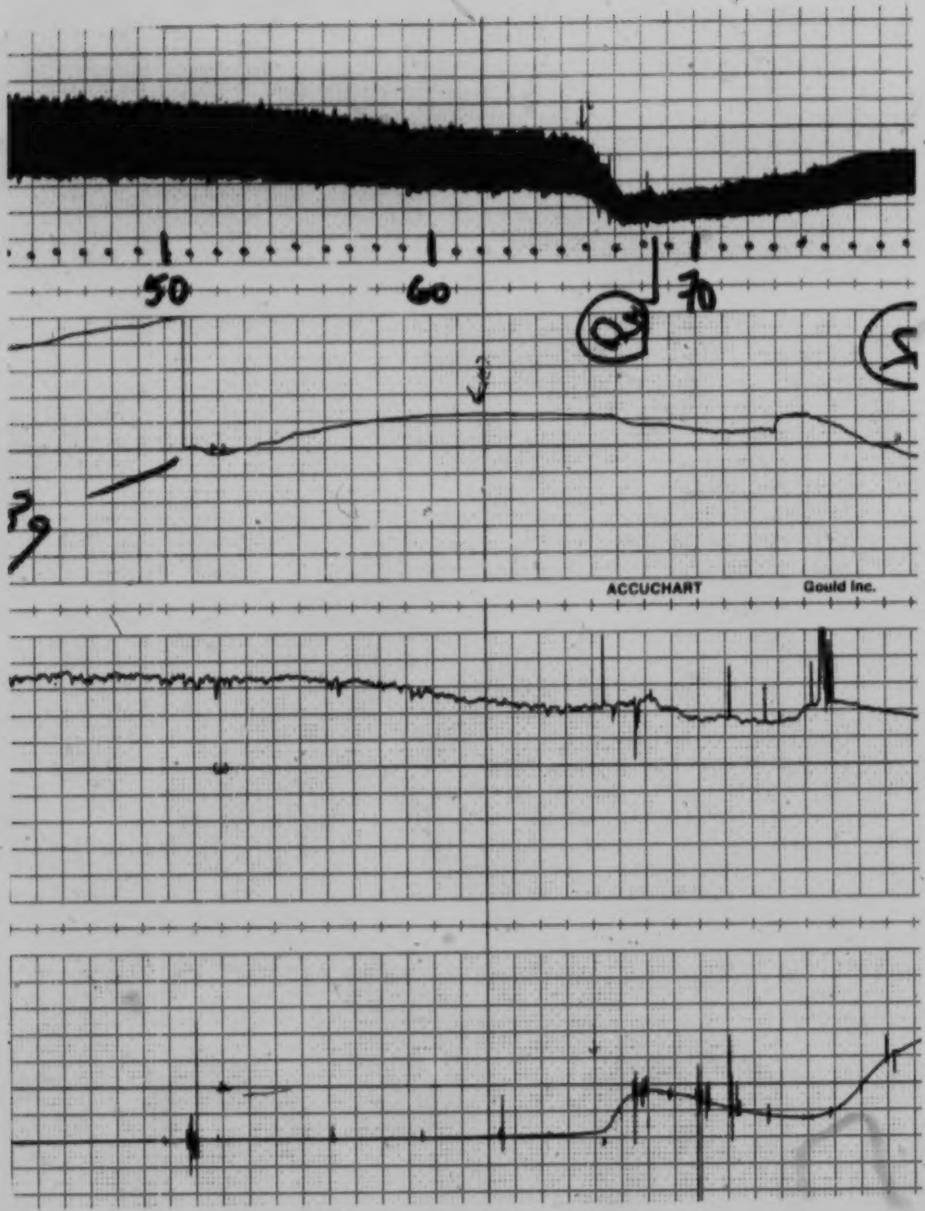


Figure 11. Acute cardiovascular collapse (ACC) after total body irradiation (TBI).

Figure 12. Reproduction of the chart recording obtained for a 110 day old rabbit which experienced ACC after TBI with 1200 cGy. The bottom tracing is of aortic blood pressure, and at 65 minutes (arrow) following TBI the acute drop began. The vertical scale is from 0 to 200 mm Hg. The time scale for the entire chart recording is indicated in minutes from the beginning of irradiation, just below the pressure tracing. The second tracing from the bottom is the heart rate, and the vertical scale is from 100 to 500 BPM. The third tracing is of rectal temperature, and the scale is from 36 to 44 degrees centigrade. The top tracing is of ear temperature, and the scale goes from 24 to 40 degrees centigrade. An important difference between this recording and that in Figure 11 is that with this rabbit the heart rate rose during ACC, rather than having declined (see text, Results III A).

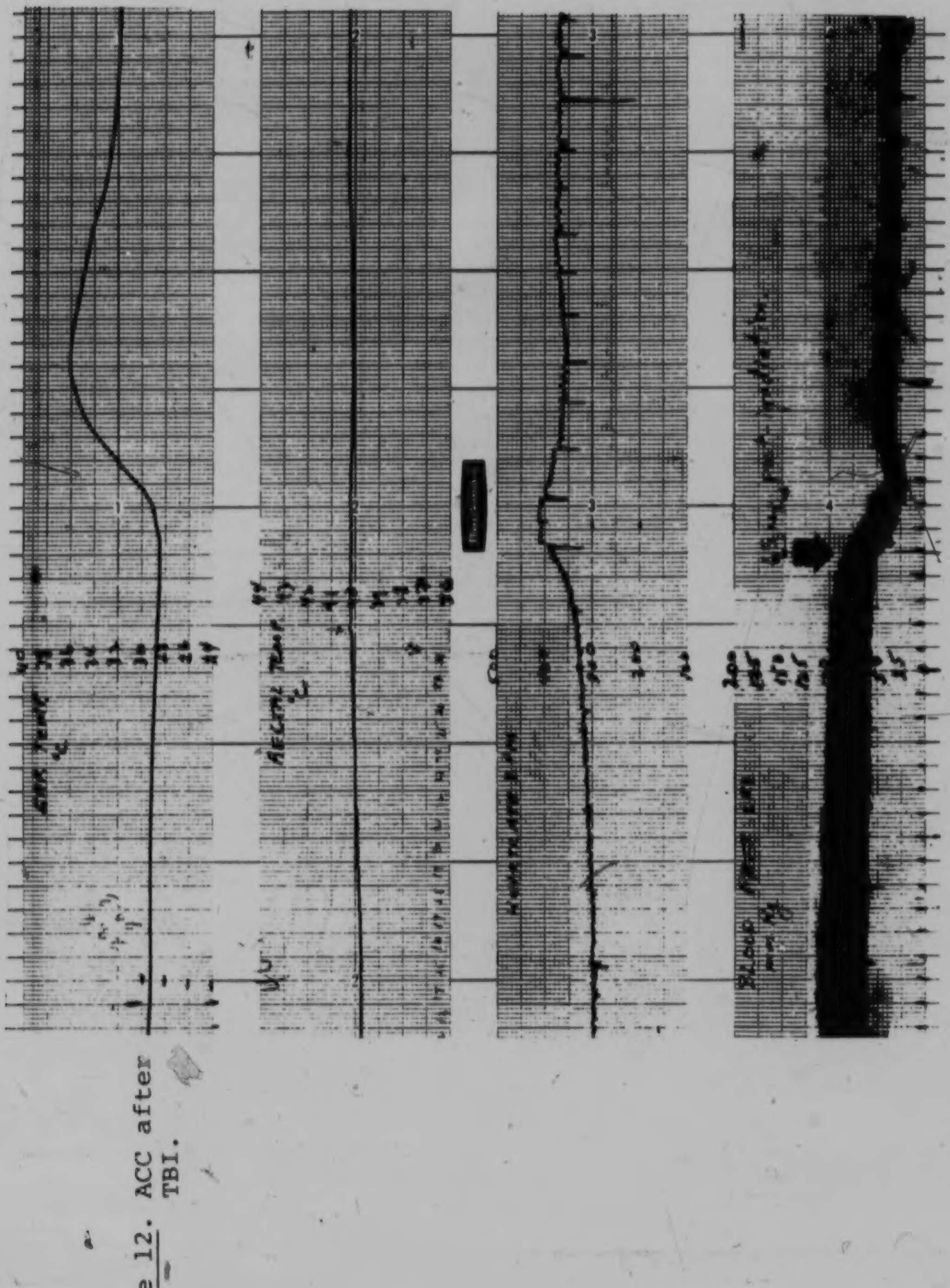


Figure 12. ACC after TBI.

Figure 13. Time versus pressure chart containing two bands which depict the acute (ACC) and the deliberate response to TBI. The vertical scale is pressure in mm Hg, and the horizontal scale indicates the time in minutes after the beginning of TBI. The hatched band was produced by first averaging the systolic and diastolic pressures at various times after irradiation for 75 40 to 100 day old rabbits which exhibited ACC. The resultant values were plotted on the chart, and the systolic points connected with a line, as were the diastolic points. The area between the systolic and diastolic lines was filled in with hatching. The hatched circles represent the averaged lowest systolic and diastolic pre-irradiation pressures for the ACC group. The dotted band was produced in the same way as the hatched band, using averaged systolic and diastolic values for 25 deliberate responders aged 40 to 100 days. The open circles represent the averaged lowest pre-irradiation systolic and diastolic pressures for the deliberate group. For both bands, some of the rabbits in the group did not produce pressure records for all the times shown on this figure. This was due in some cases to intermittent clotting, delay in reconnection after TBI, equipment problems etc. Hence, the rabbits which did produce data for those times were used.

Fig. 13: Response to TBI; 40–100 Days

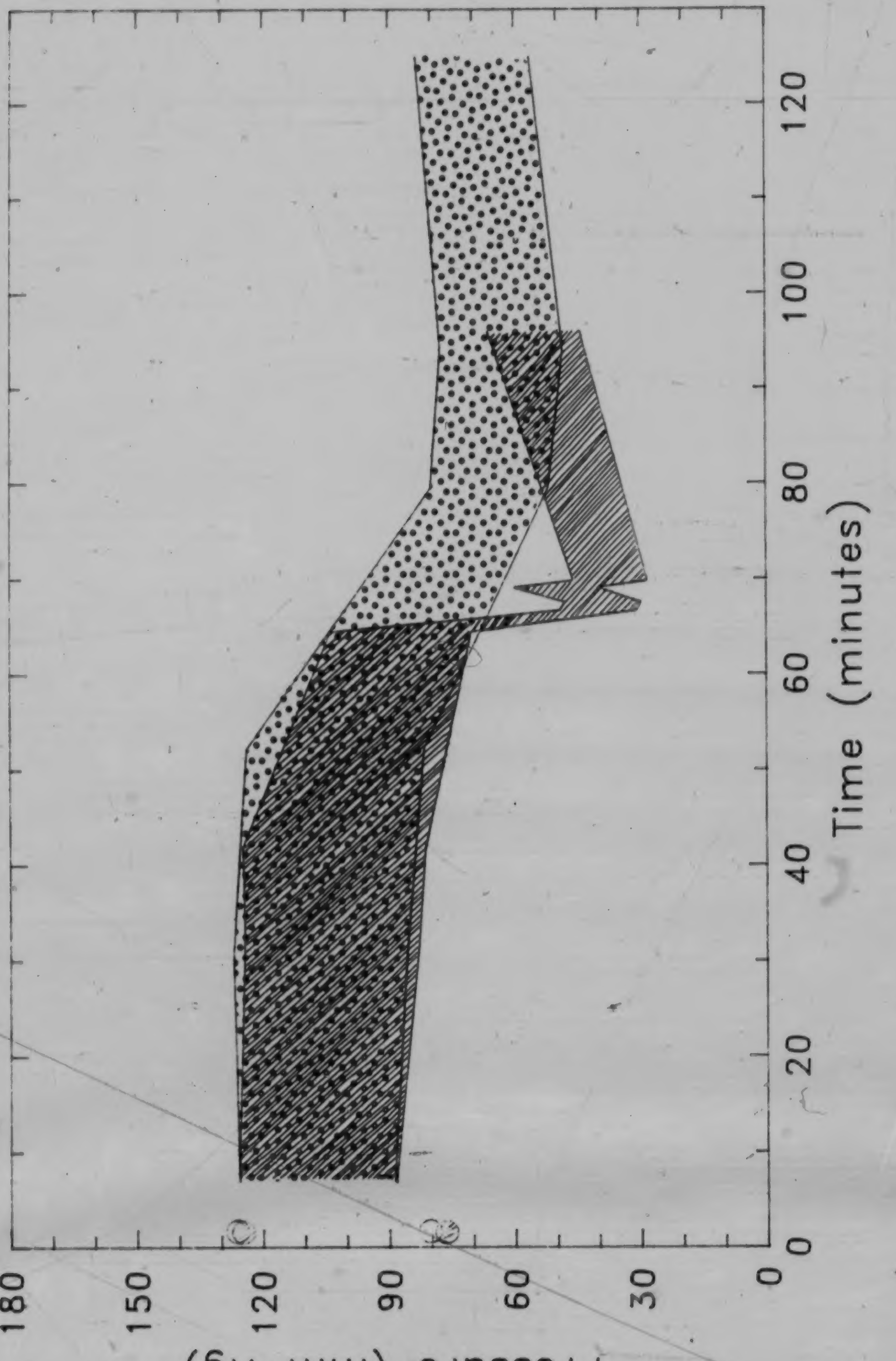


Figure 14. Reproduction of a chart recording obtained for a 64 day old rabbit which displayed the deliberate response after TBI with 1191 cGy at 1160 cGy/minute. The bottom tracing is the aortic blood pressure, and the vertical scale is from 0 to 200 mm Hg. Horizontally, each major chart grid division represents one minute of recording time, and the recording begins at about 7 minutes after TBI. The second tracing from the bottom is the heart rate, and the scale goes from 100 to 500 BPM. The third trace is the rectal temperature, and the vertical scale is either from 36 to 44 degrees C, or from 37 to 41 degrees C. The top tracing is the ear temperature, and the scale is from 24 to 40 degrees C.

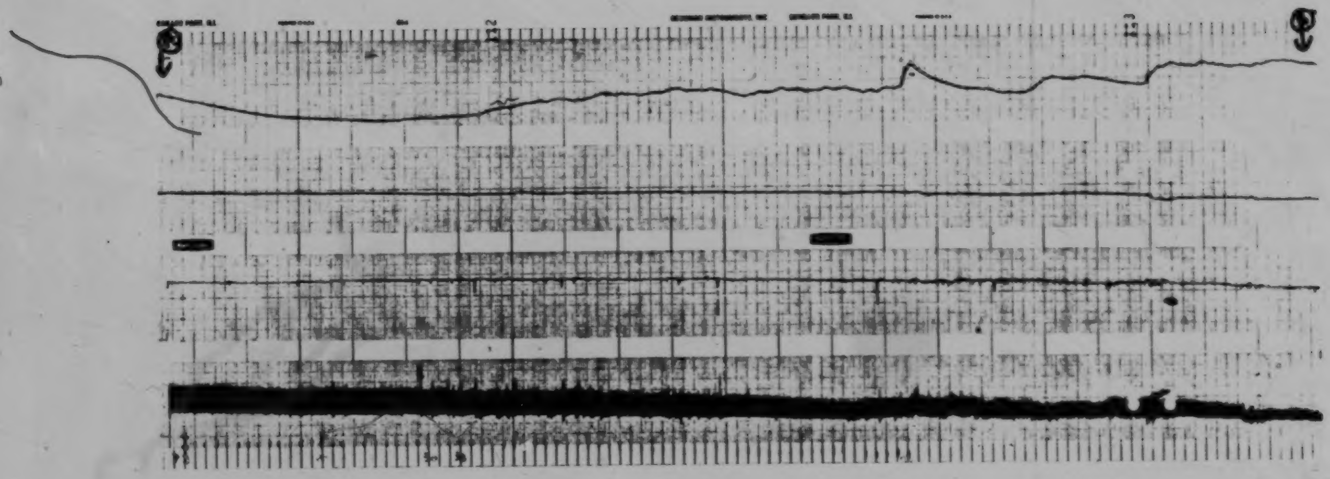


Figure 14. The deliberate response to TBI.

Figure 15. Chart recording for which arterial blood pressure was recorded using the tail cuff and the cannula methods simultaneously. The 62 day old rabbit exhibited ACC after TBI with 1038 cGy. The time scale for the entire chart record is indicated in minutes after the beginning of irradiation, just below the bottom blood pressure tracing. The lowest tracing was obtained using the cannula, and the vertical scale is from 0 to 200 mm Hg. The second tracing from the bottom was produced using the tail cuff method, and the vertical scale goes from 0 to 200 mm Hg. The topmost tracing is of ear temperature, and the vertical scale is 24 to 40 degrees centigrade. The acute pressure drop commenced at roughly 64 minutes after TBI. The chart paper was run rapidly so that tail cuff derived pressures would be readable.

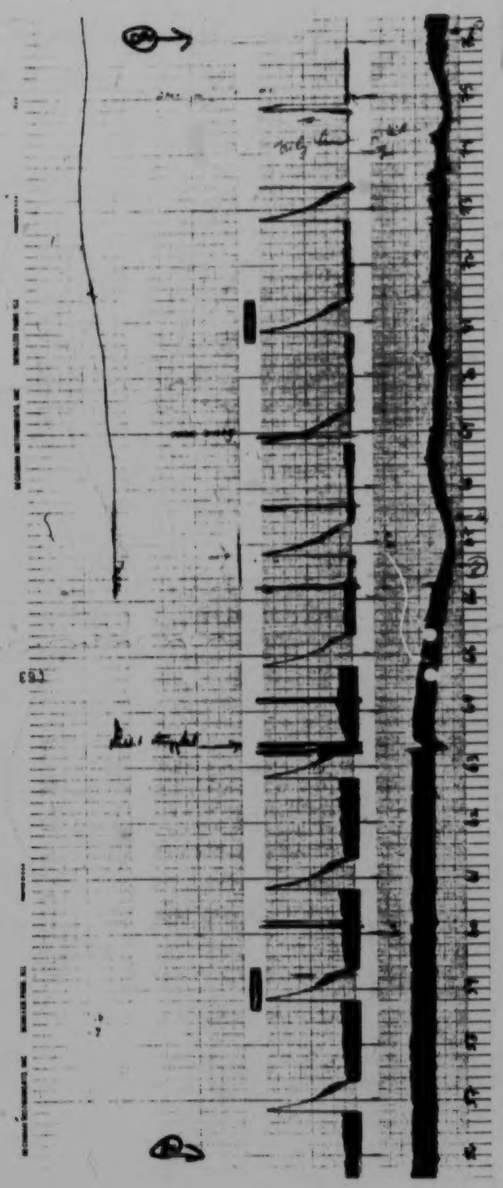
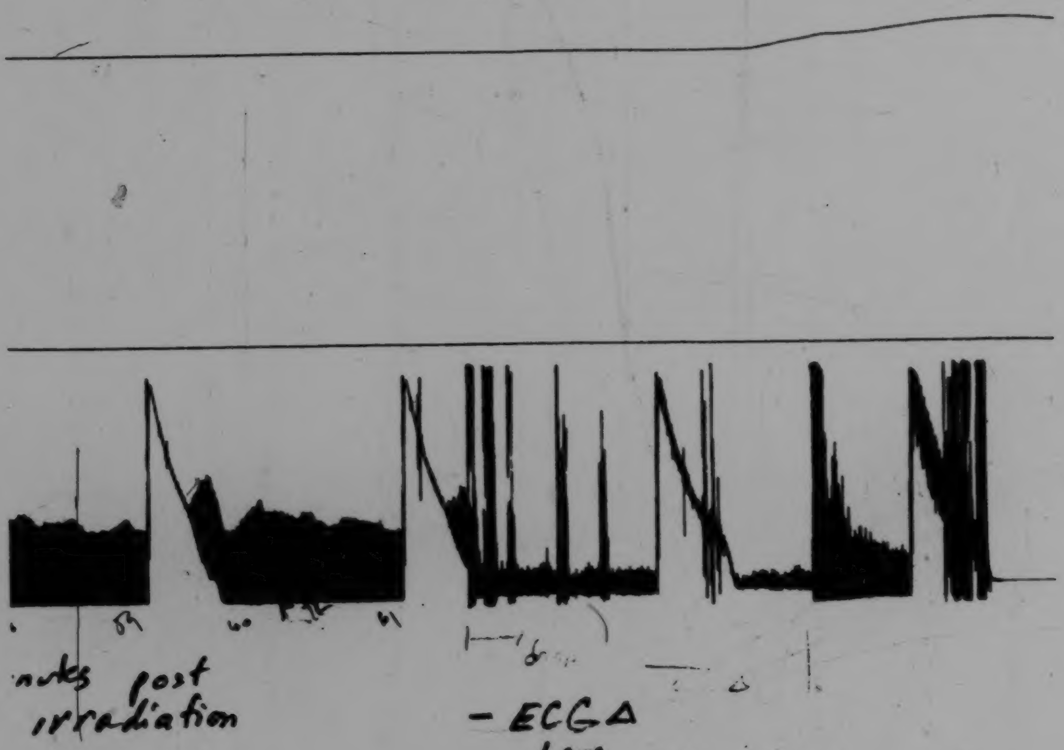


Figure 15. Cannula and tail cuff methods together in an acute responder.

Figure 16. Reproduction of a chart recording obtained for an 84 day old rabbit which displayed ACC after TBI with 1278 cGy and which was monitored with the tail cuff method. The tracing in the lower third of the figure is the tail pulse superimposed on cuff air pressure. The time in minutes from the beginning of TBI is indicated just beneath the pulse trace. The pulse amplitude declined very rapidly at about 61½ minutes after TBI, and the systolic arterial pressure was not readable after this time. The ear temperature is shown in the top tracing, and a rise is evident just after TBI. The ear temperature increase seems late and slow, but this is due to the relatively fast chart feed rate, 25 mm/minute, compared with 5 mm/minute for cannulated rabbits. The blood pressure vertical scale goes from 0 to 200 mm Hg, while the ear temperature scale is from 24 to 40 degrees C. Note that a change in the ECG was observed, and the rabbit became flaccid.



wks post irradiation

- ECG Δ here
- rabbit flaccid.

Figure 16. ACC in a tail cuff monitored rabbit.

Figure 17. Graph showing how the steepness of the post-TBI blood pressure decline varied with age. The vertical axis comprises slope divided by age, and the horizontal axis is age; both axes are scaled to $\log_{10}$. The middle line running through the points is the regression, and the two outer lines encompass the data which were included in the regression calculation. Although it may not be readily apparent due to the logarithmic scale, the cluster of 40 to 100 day old rabbits near the bottom of the plot comprise a group which is well removed from the remainder of the young rabbits. Even if these points were included in the regression, the slope of the line would be relatively unchanged. It can be seen that the greatest proportion of steep responders (acute responders) resides in the 40 to 100 day group. Older rabbits tend not to exhibit a very steep pressure decline after TBI.

Figure 17: Slope of Blood Pressure Decline According to Age

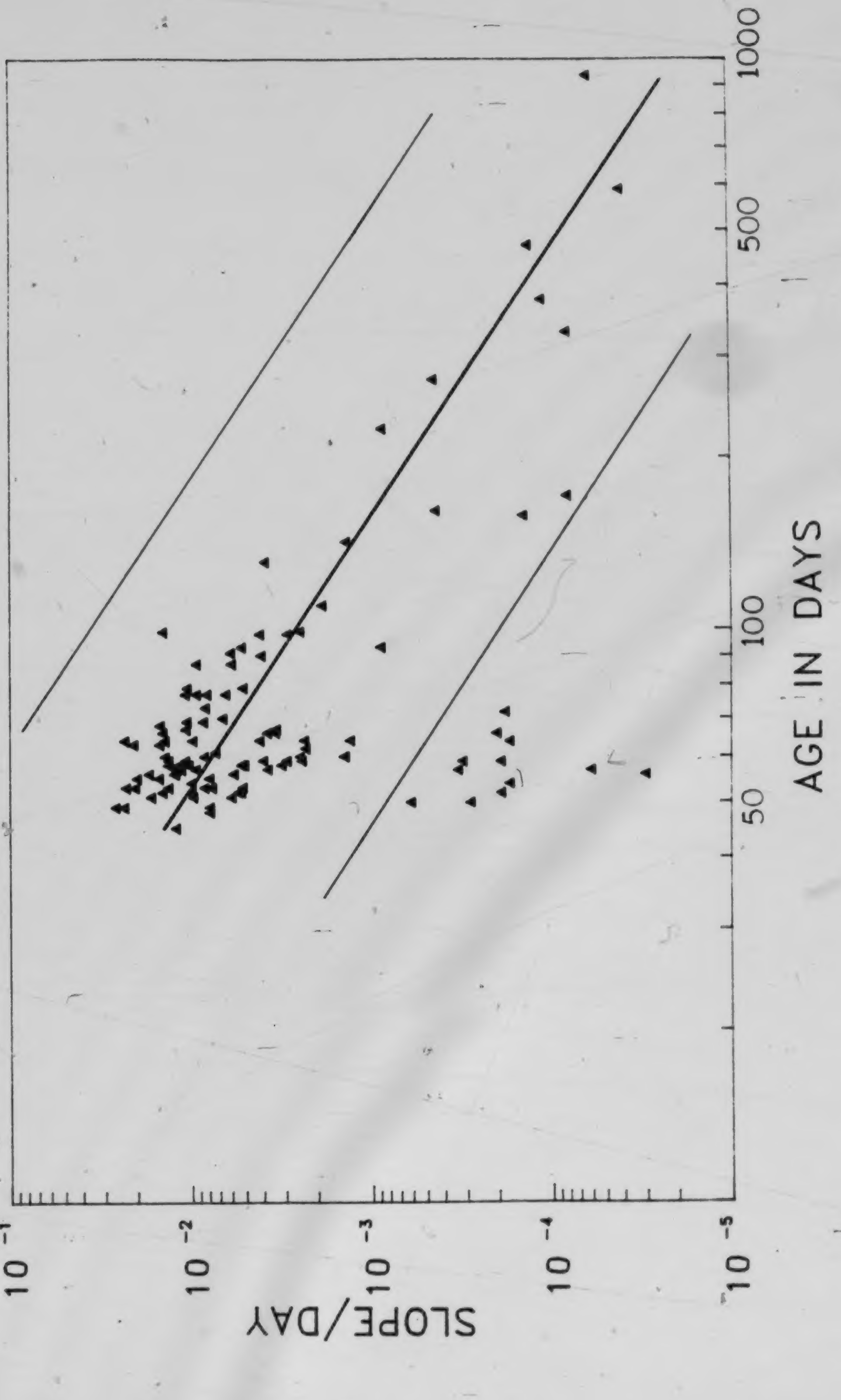


Figure 18. Graph showing that rabbits aged 40 to 100 days can be divided into two groups on the basis of the slope of the post-TBI blood pressure decline. The solid line is the cumulative number of rabbits from left to right, arranged according to increasing slope, originally plotted on probability paper. Note the sharp point of inflection, dividing the line into two distinct segments; this proves the bimodal character of the population. The general location of the breakpoint is not arbitrary;—it represents the true nature of the data when plotted on probability paper. The bars indicate the number of rabbits in various slope classes, and have been included to provide a pictorial impression of the slope distribution. The graph shows that the separation of the cardiovascular response into two types, acute and deliberate, is valid. The righthand axis is not drawn quite to scale; the "1" is too high. The axis was drawn this way to facilitate reading of the graph.

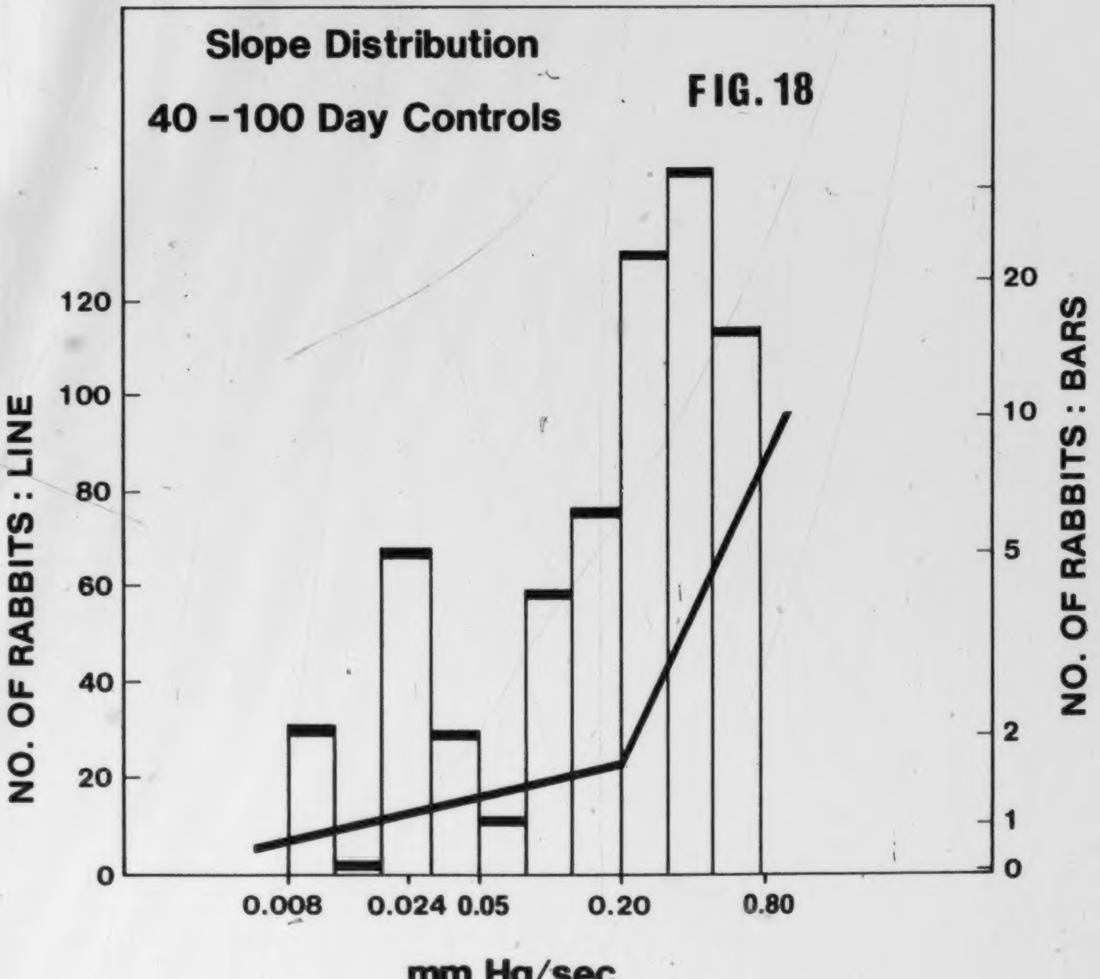


FIG. 18

Slope Distribution

40 -100 Day Controls

Figure 19. The cardiovascular response to TBI of rabbits aged 100 to 935 days. The systolic and diastolic pressures at various times after irradiation were averaged for 11 deliberate responders. The black circles denote the averaged, lowest systolic and diastolic pre-irradiation pressures for this group. The vertical axis is pressure in mm Hg, and the horizontal axis is time in minutes after the beginning of TBI.

Fig. 19: Response to TBI; 100–935 Days

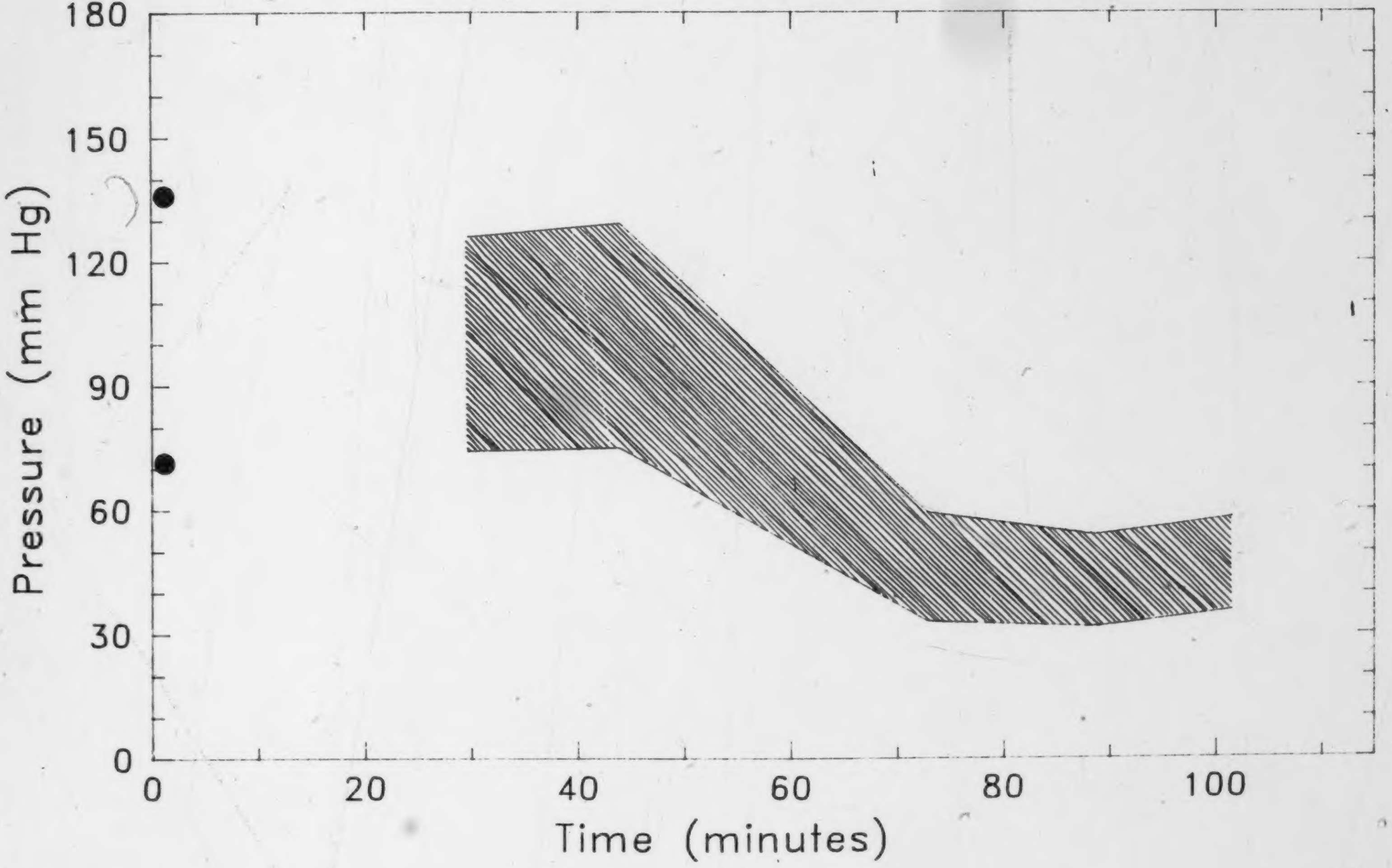


Figure 20. Graph of the frequency of ACC for various dose categories. The vertical axis is frequency of ACC in percent, and the horizontal axis is the radiation dose in Grays (Gy), scaled to the $\log_{10}$. This graph contains dose ranges which are unequal, so dose was placed on a logarithmically scaled axis to equalize the apparent class widths, thereby aiding visual interpretation. Also, the graph was produced by pooling data from cuff and cannulated rabbits, and it should be noted that the higher dose ranges include relatively more cuff monitored animals for which detection of ACC was more difficult.

Fig. 20: Frequency of Acute Hypotension vs. TBI Dose

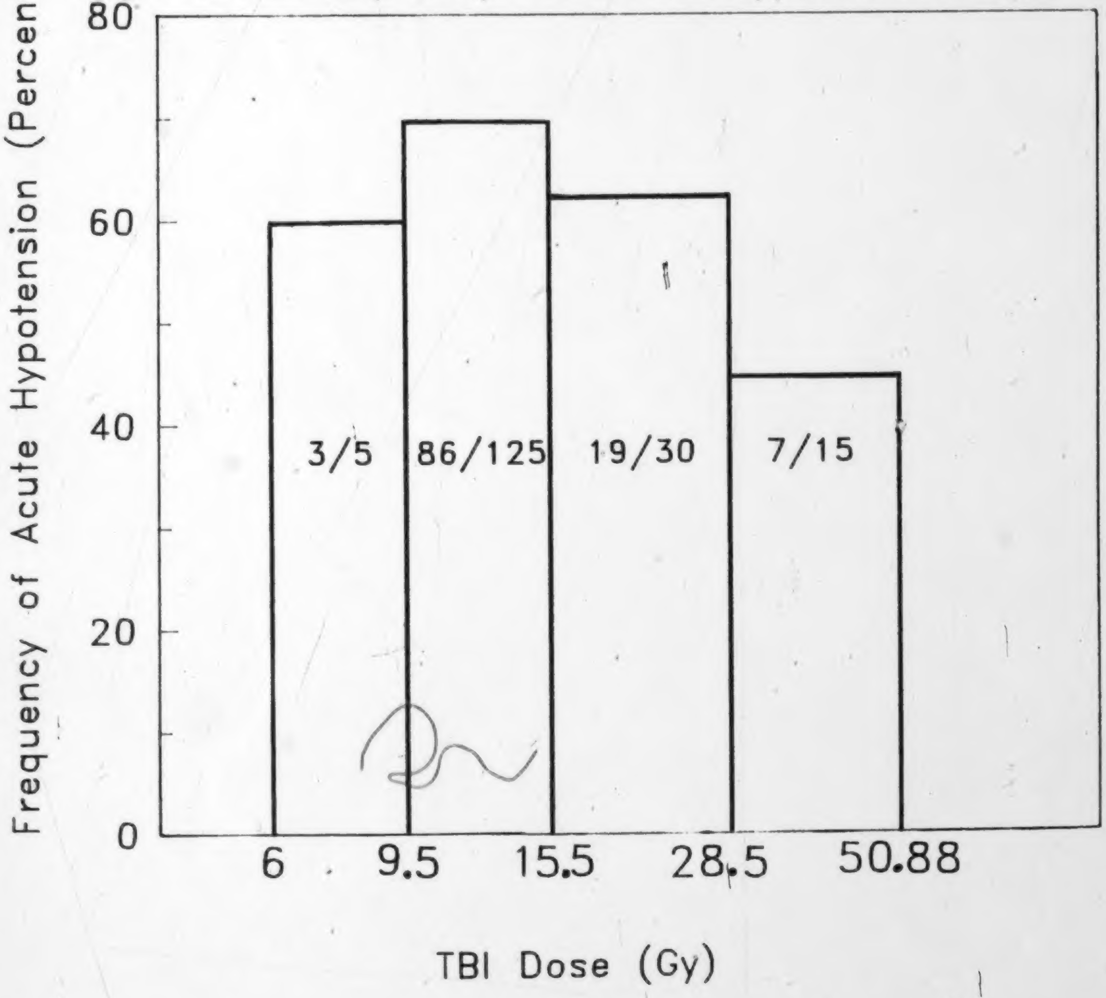


Figure 21. Bar graph showing the average percent total hypotension, and the frequency of ACC for various treatment categories. Only rabbits which were cannulated in the dorsal aorta, and which were variously irradiated with between 9.5 and 15.5 Gy are included. The vertical axis separates the treatment groups, and the horizontal axis indicates percent. The hatched bars show the average percent total decline in mean aortic pressure for all the rabbits, acute and deliberate responders together, for each treatment. The standard error of the mean is indicated on each hatched bar. The solid bars indicate the proportion of the total number of animals tested in each treatment group which displayed ACC. The number of rabbits tested in each treatment group is indicated at the right of every solid bar.

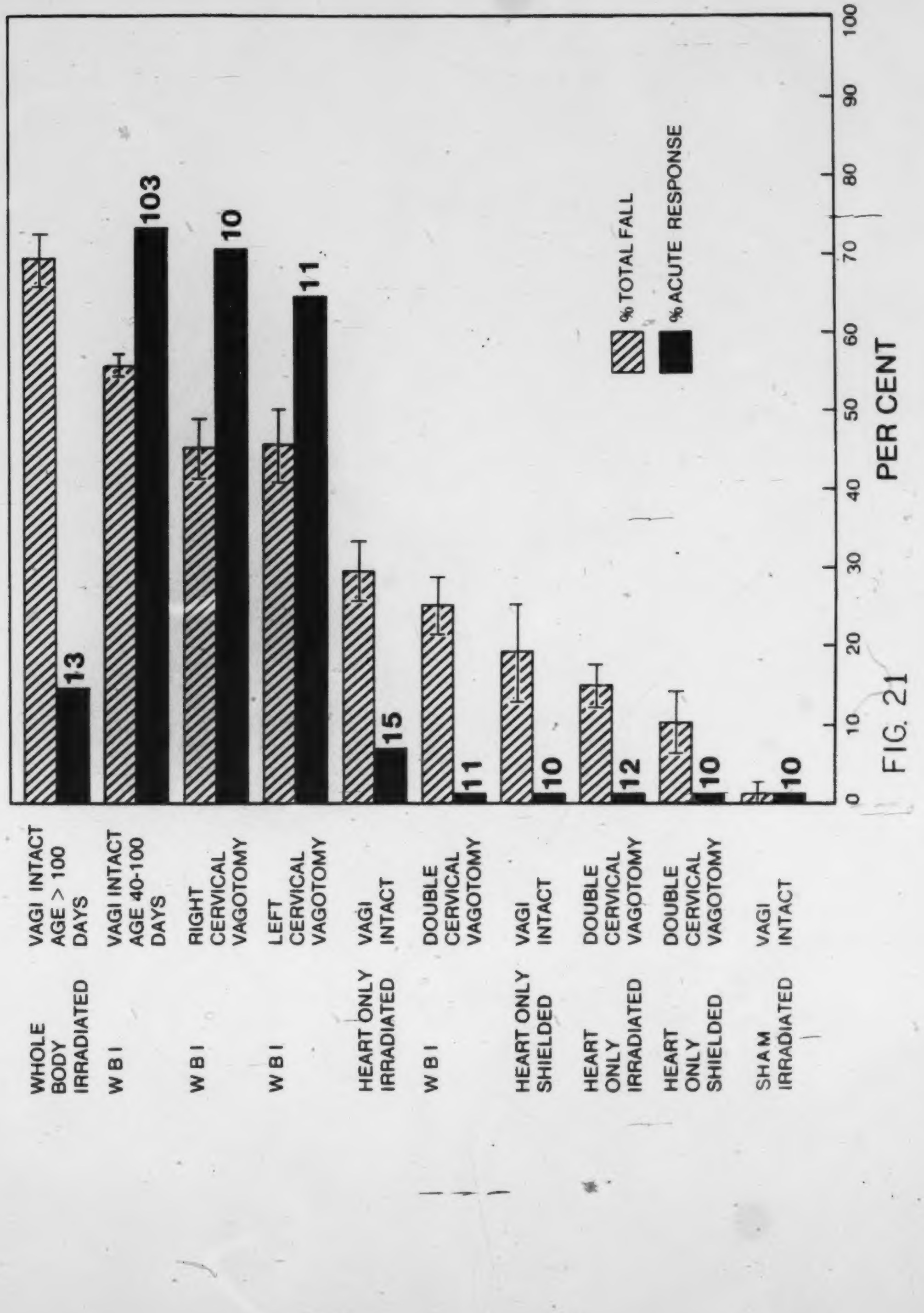

WHOLE BODY IRRADIATED
VAGI INTACT AGE > 100 DAYS
13
W B I
VAGI INTACT AGE 40-100 DAYS
103
W B I
RIGHT CERVICAL VAGOTOMY
10
W B I
LEFT CERVICAL VAGOTOMY
11
HEART ONLY IRRADIATED
VAGI INTACT
15
W B I
DOUBLE CERVICAL VAGOTOMY
11
HEART ONLY SHIELDED
VAGI INTACT
10
HEART ONLY IRRADIATED
DOUBLE CERVICAL VAGOTOMY
12
HEART ONLY SHIELDED
DOUBLE CERVICAL VAGOTOMY
10
SHAM IRRADIATED
VAGI INTACT
10
▨ % TOTAL FALL
■ % ACUTE RESPONSE
0
10
20
30
40
50
60
70
80
90
100
PER CENT

FIG. 21

Figure 22. The cardiovascular response to selective heart irradiation of rabbits aged 40 to 100 days. The dotted band was produced by averaging the systolic and diastolic pressures at various times after irradiation for 14 deliberate responders. The open circles indicate the averaged, lowest systolic and diastolic pre-irradiation pressures for this group. The hatched band shows the response of the one acute responder. The hatched circles denote the lowest systolic and diastolic pre-irradiation pressures for this animal. The vertical axis is pressure in mm Hg, and the horizontal axis indicates the time in minutes after the beginning of heart irradiation.

Fig. 22: Selective Heart Irradiation

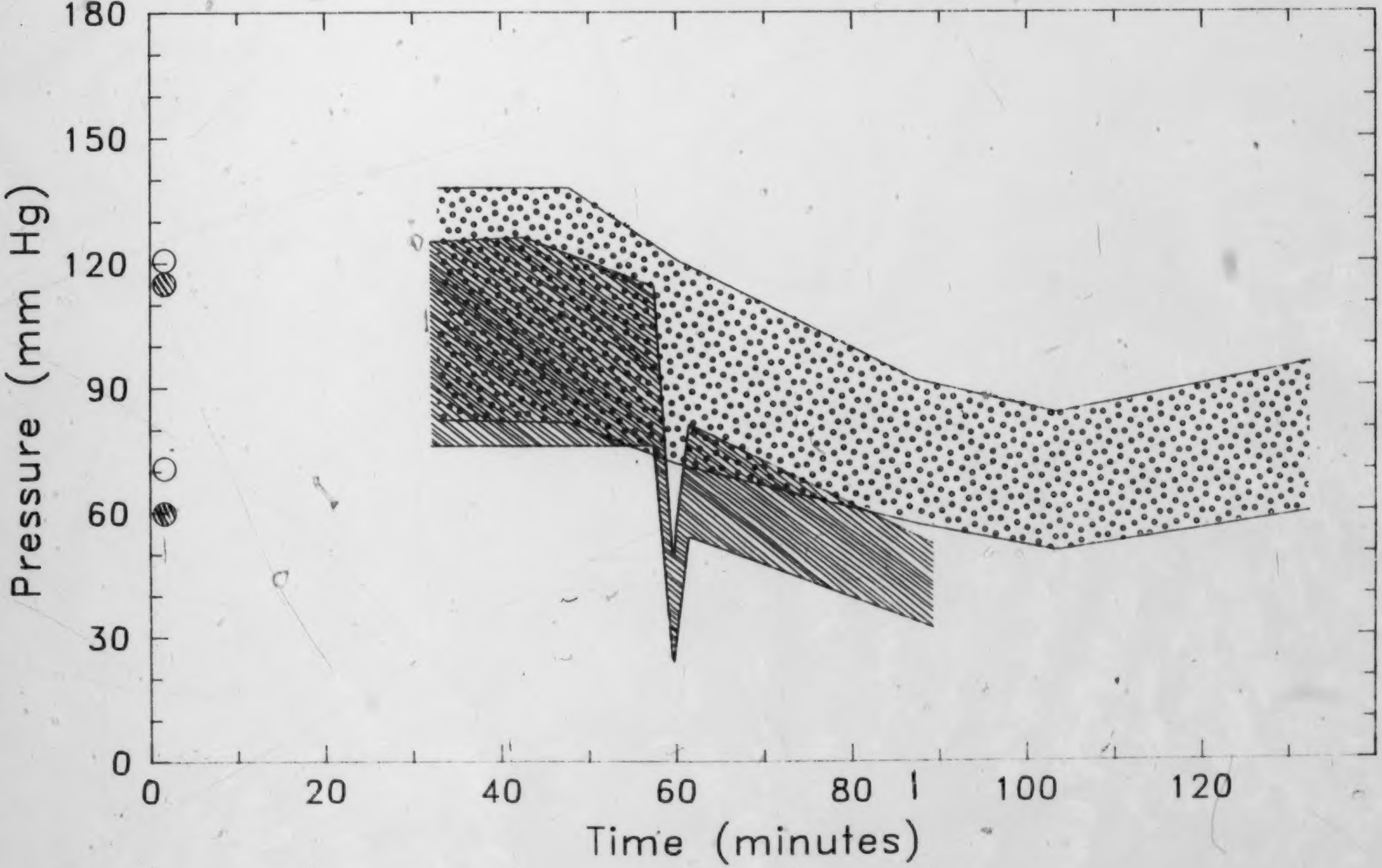


Figure 23. The cardiovascular response to TBI with the heart region shielded, in rabbits aged 40 to 100 days. The dotted band was produced by averaging the systolic and diastolic pressures at various times after irradiation for 9 deliberate responders. The open circles denote averaged, lowest systolic and diastolic pre-irradiation pressures for this group. The hatched band shows the response of the one acute responder. The hatched circles indicate averaged, lowest systolic and diastolic pre-irradiation pressures for this animal. The vertical axis is pressure in mm Hg, and the horizontal axis is the time in minutes from the beginning of TBI.

Fig. 23: TBI with Heart Shielded

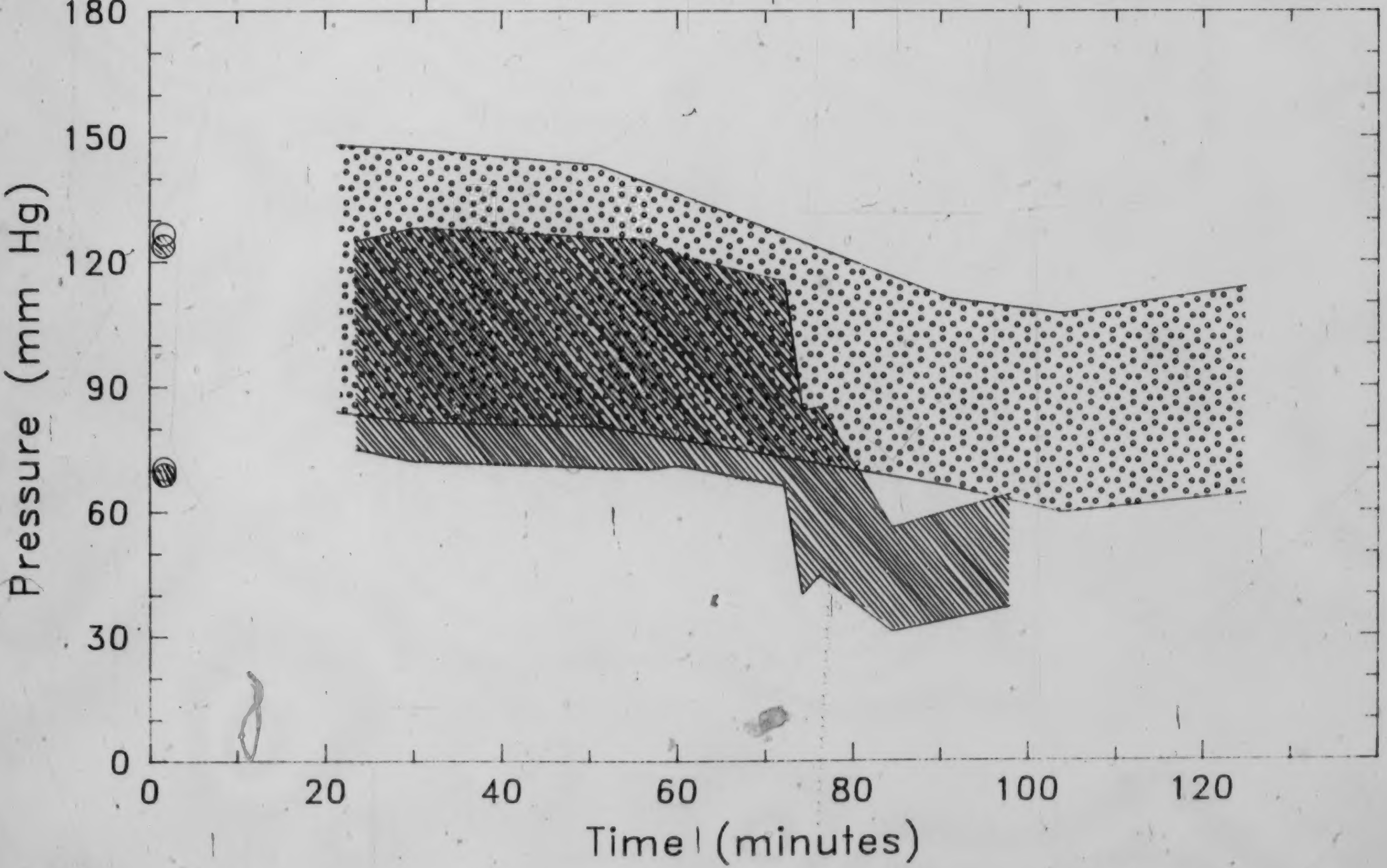


Figure 24. The cardiovascular response of 40 to 100 day old rabbits which were bilaterally vagotomized and then subjected to TBI. The band was produced by averaging the systolic and diastolic pressures at various times after irradiation, for the 11 rabbits. The black circles denote averaged, lowest systolic and diastolic pre-irradiation pressures for this group. The vertical axis is pressure in mm Hg, and the horizontal axis indicates the time in minutes from the beginning of TBI.

Fig. 24: Double Vagotomy

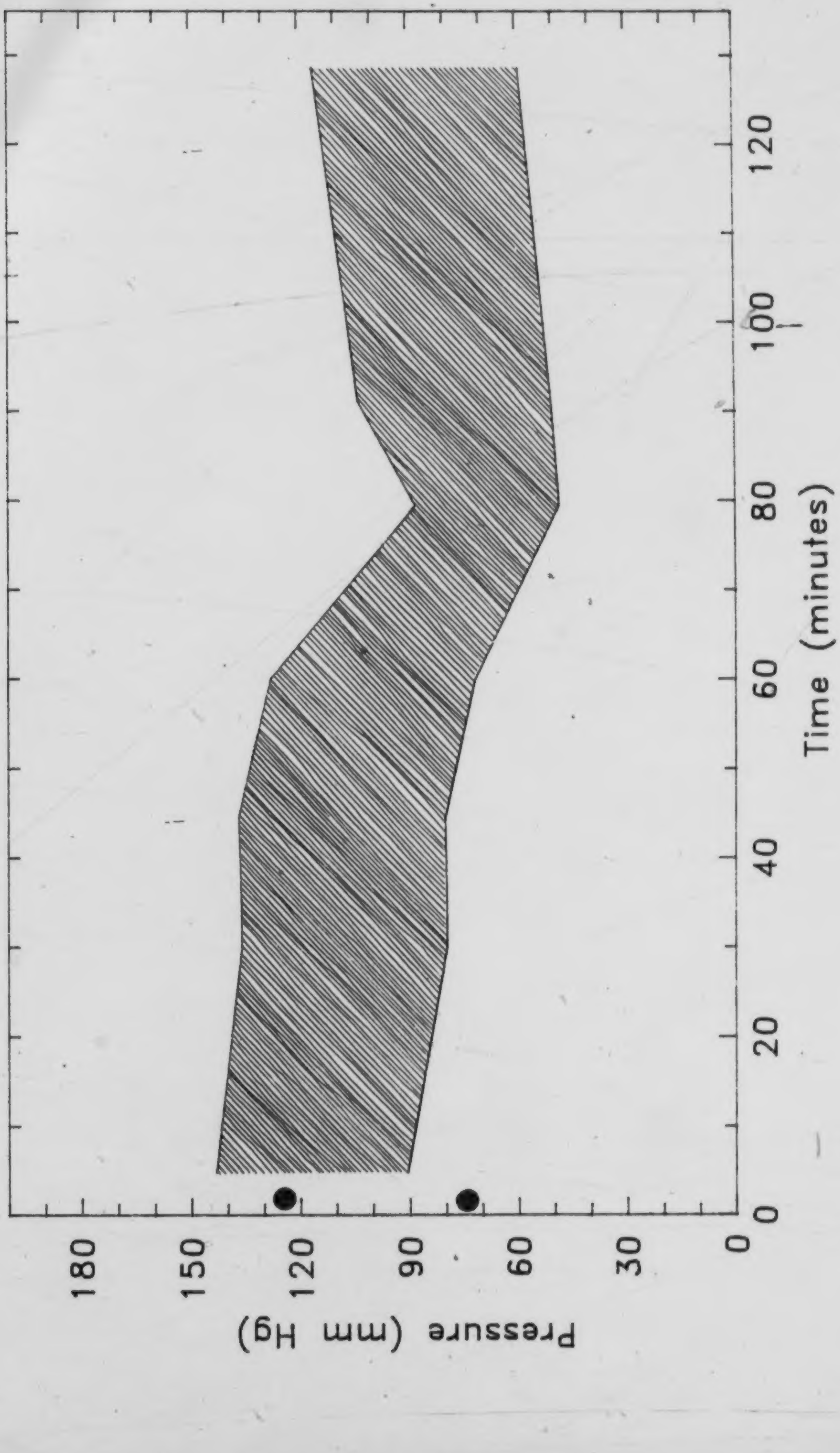


Figure 25. The cardiovascular response of 40 to 100 day old rabbits which were bilaterally vagotomized and then subjected to heart irradiation. The band was produced by averaging the systolic and diastolic pressures at various times after irradiation, for the 12 rabbits. The black circles indicate averaged, lowest systolic and diastolic pre-irradiation pressures for this group. The vertical axis is pressure in mm Hg, and the horizontal axis indicates the time in minutes after the beginning of heart irradiation.

Fig. 25: Double Vagotomy and Heart Irradiation

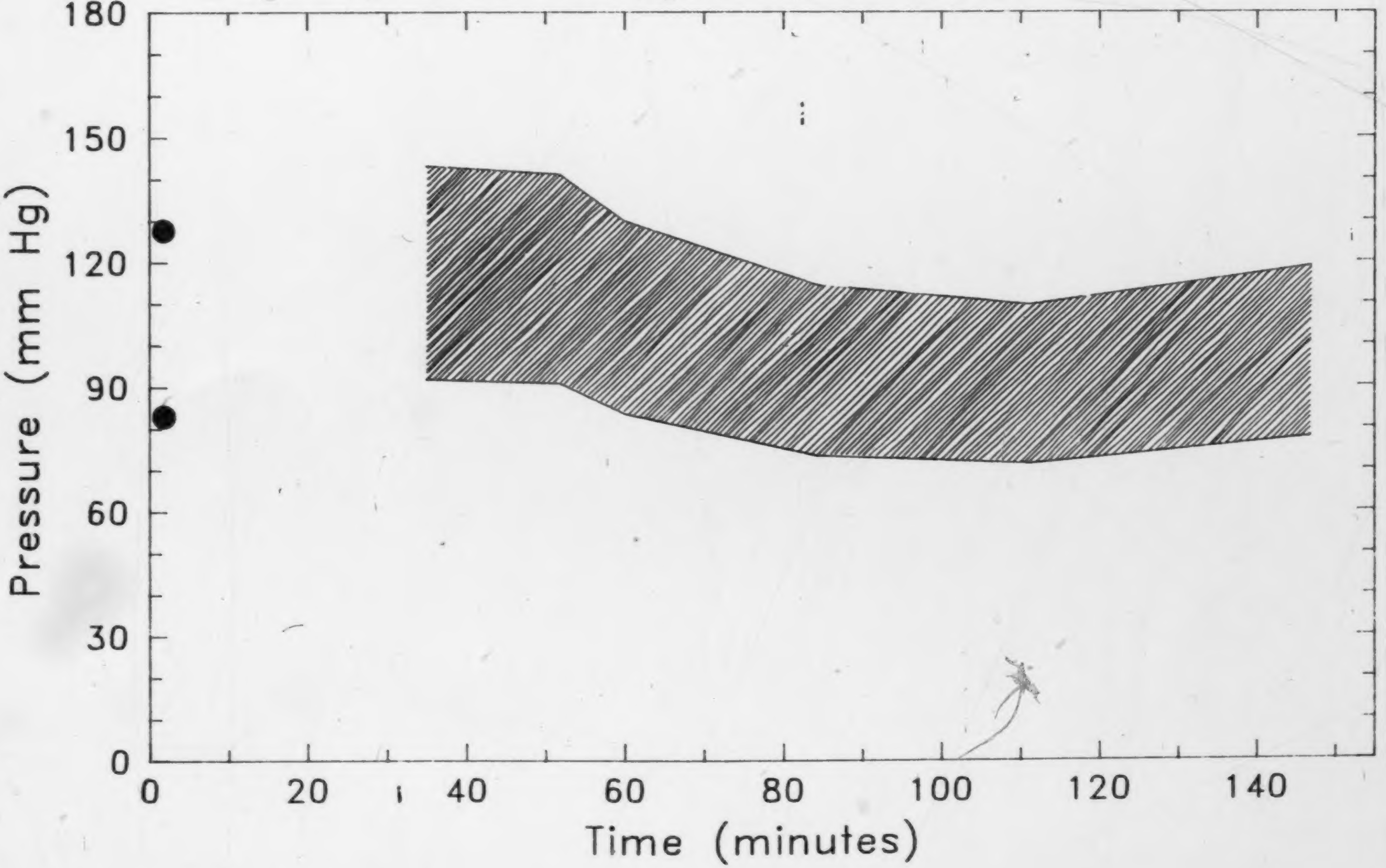


Figure 26. The cardiovascular response of 40 to 100 day old rabbits which were bilaterally vagotomized and then subjected to TBI with heart shielding. The band was produced by averaging the systolic and diastolic pressures at various times after irradiation, for the 10 rabbits. The black circles indicate averaged, lowest systolic and diastolic pre-irradiation pressures for this group. The vertical axis is pressure in mm Hg, and the horizontal axis indicates the time in minutes from the beginning of TBI.

Fig. 26: Double Vagotomy with Heart Shielding

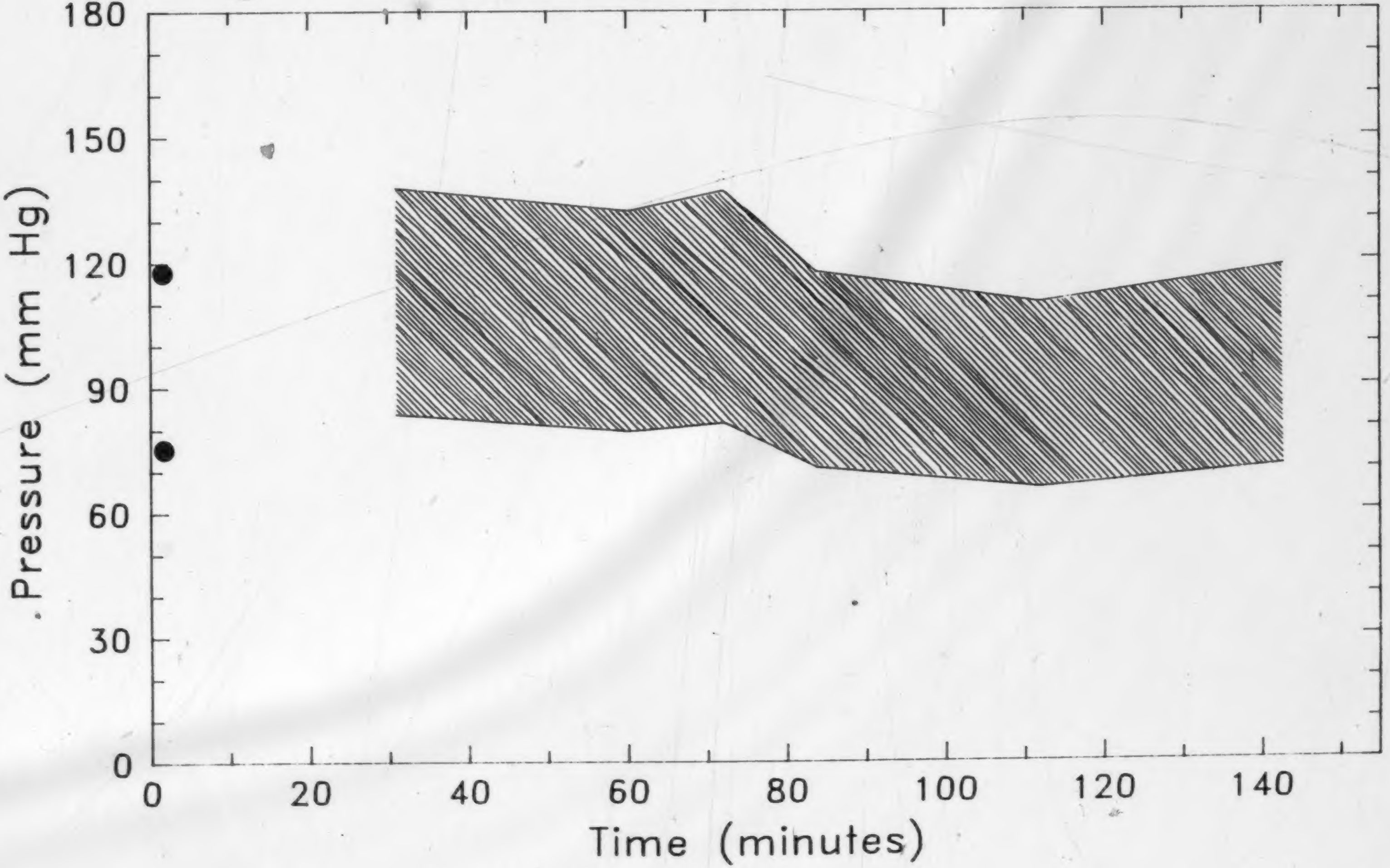


Figure 27. The effect of vagotomy on radiation hypotension. The horizontal axis shows the number of vagi which were transected prior to three different types of irradiation. These exposures were: (1) TBI, (2) TBI with heart shielding, and (3) heart irradiation. The vertical axis indicates the lowest post-irradiation mean aortic pressure, averaged for each treatment group of rabbits, and expressed as the percentage of the lowest pre-irradiation mean pressure. The graph shows that for each type of irradiation, double vagotomy reduced the extent of post-irradiation hypotension.

Fig. 27: Effect of Vagotomy on Irradiation Hypotension

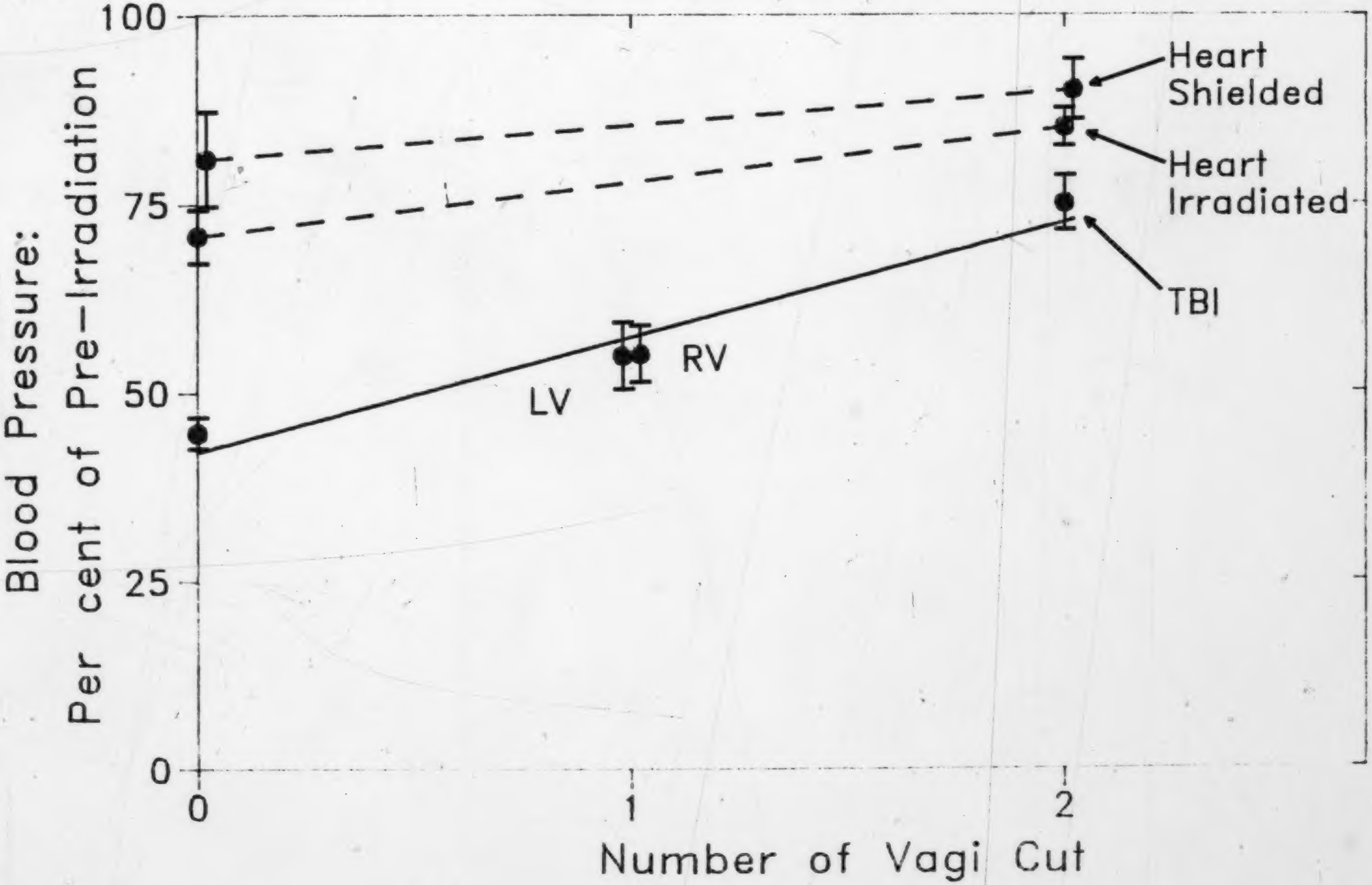


Figure 28. The per cent change in each frequency band of the vagal power spectrum after TBI, in six 40 to 100 day old rabbits which displayed the acute response. The figure was prepared by first subtracting the microvolt value in each band of a pre-irradiation power spectrum photograph, from the microvolt values in the corresponding bands belonging to power spectrum photographs taken at three different times after TBI. The resultant values for each frequency band were then divided by the pre-irradiation values, and each quotient was multiplied by 100 to yield per cent change. This was done for each of the six rabbits. Then, the per cent change values for the six rabbits were averaged for each of the three times after TBI, and the results were plotted. Figures 28 to 31 were all basically constructed using the above approach. The symbols used on this graph are unique for each time after TBI, and the symbols for each particular time are connected with a line, as a visual aid. The vertical axis is per cent change, going from -100% to +100%, and the horizontal axis is of frequency, scaled logarithmically ($\log_{10}$). The figure legend identifies the mean post-irradiation times represented by each set of symbols, and also indicates the ranges (in brackets) associated with each mean time.

Fig. 28: Power Spectrum After TBI – Acutes

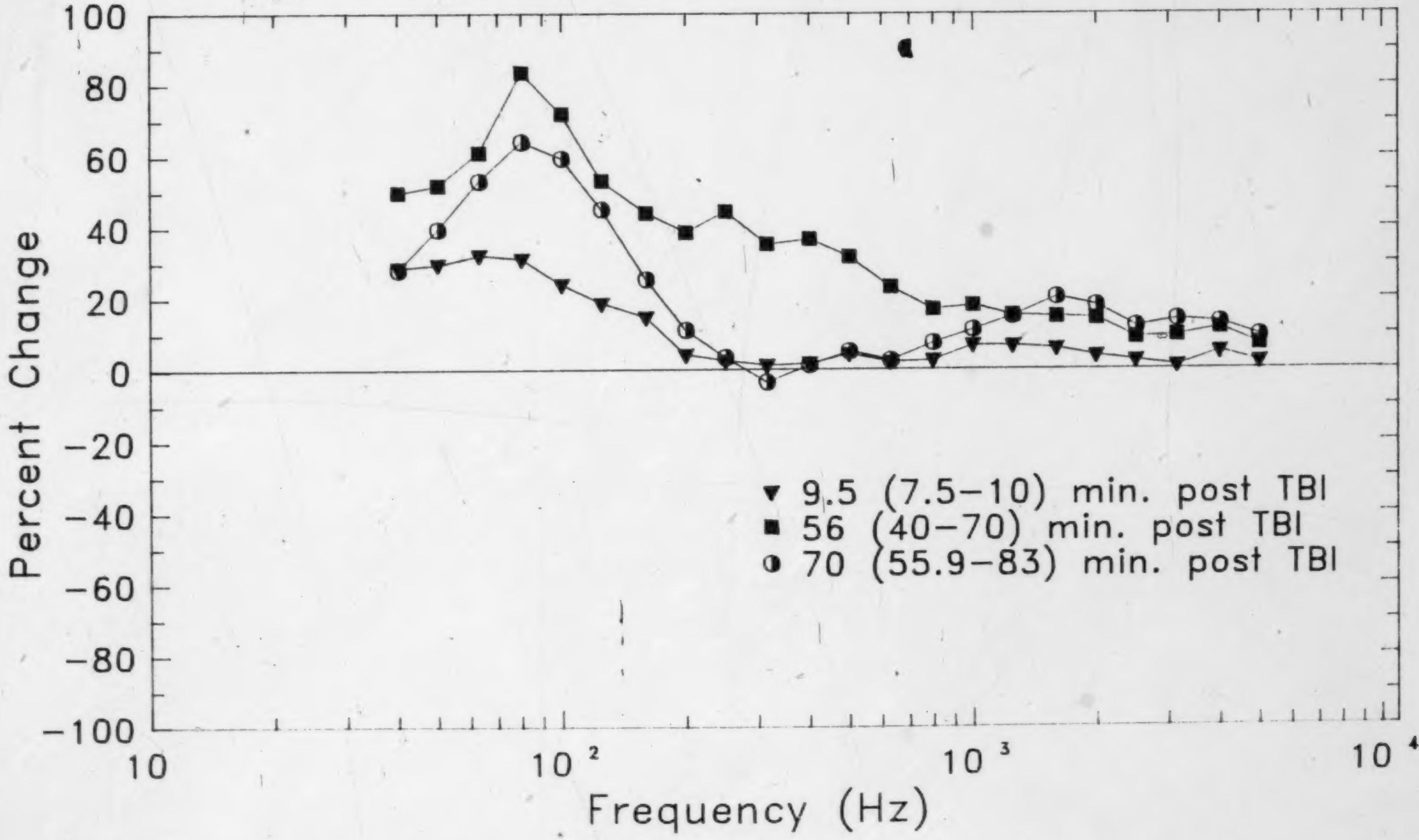


Figure 29. The per cent change in each frequency band of the vagal power spectrum after TBI, for two 40 to 100 day old rabbits which exhibited the deliberate response. The two deliberate responders used for this figure produced power spectra which contained relatively low voltage amplitudes in the range of frequencies between about 150 and 400 Hz. The per cent change values for the rabbit were averaged for each of three different times after TBI. The mean times are indicated by the figure legend, as are the individual times (in brackets) which were used to calculate the means. The vertical axis shows the per cent change, going from -100% to +100%, and the horizontal axis is of frequency, scaled logarithmically ($\log_{10}$).

Fig 29: Power Spectrum After TBI — Deliberates

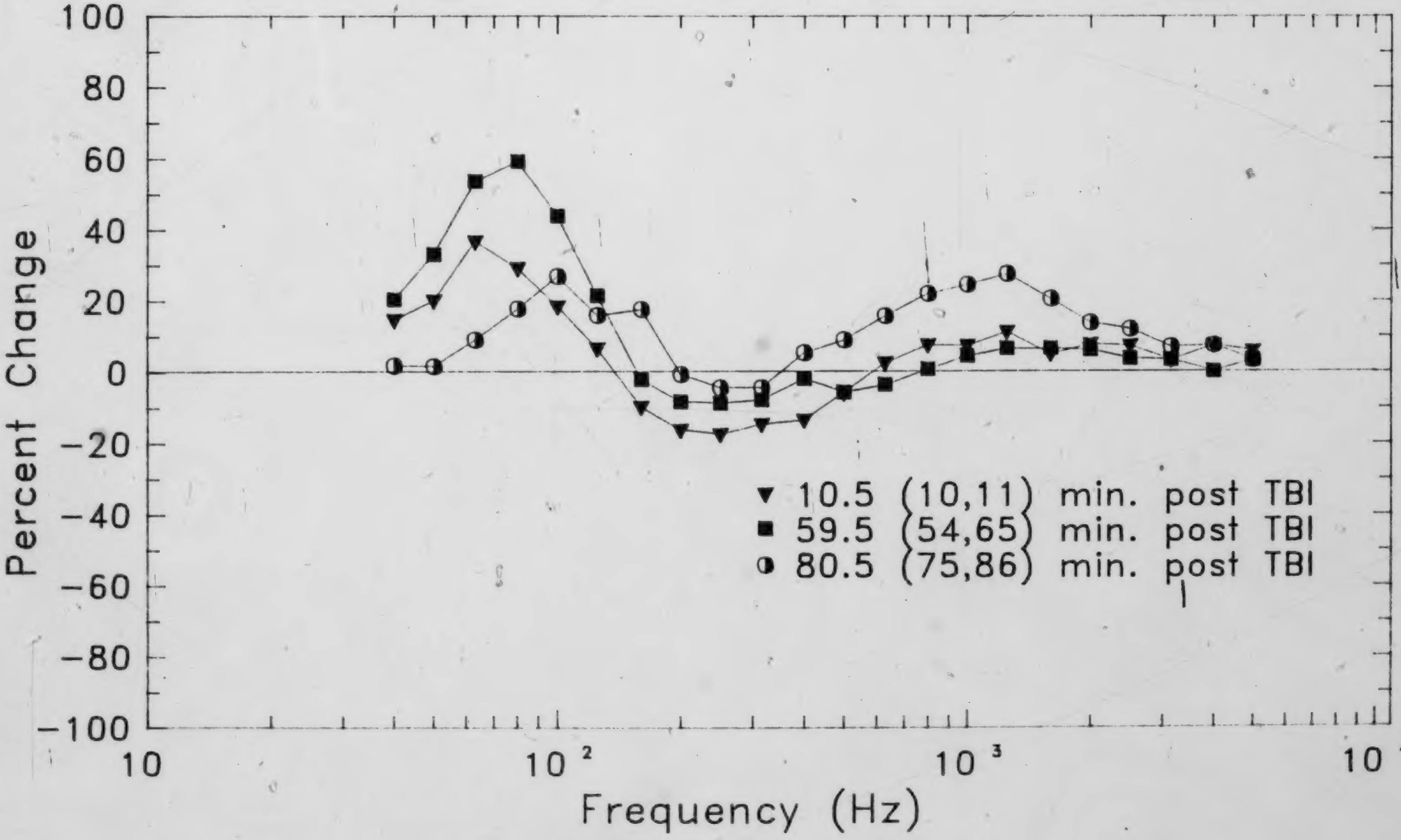


Figure 30. The per cent change in each frequency band of the vagal power spectrum after TBI, in four 40 to 100 day old rabbits which exhibited the deliberate response. The four deliberate responders used for this figure produced power spectra which contained relatively high voltage amplitudes in the range of frequencies between about 150 and 700 Hz. The per cent change values for the rabbits were averaged for each of three different times after TBI. The mean times are indicated by the figure legend, as are the associated time ranges (brackets). The vertical axis shows the per cent change, going from -100% to +100%, and the horizontal axis is of frequency, scaled logarithmically ($\log_{10}$).

Fig. 30: Power Spectrum After TBI – Deliberates

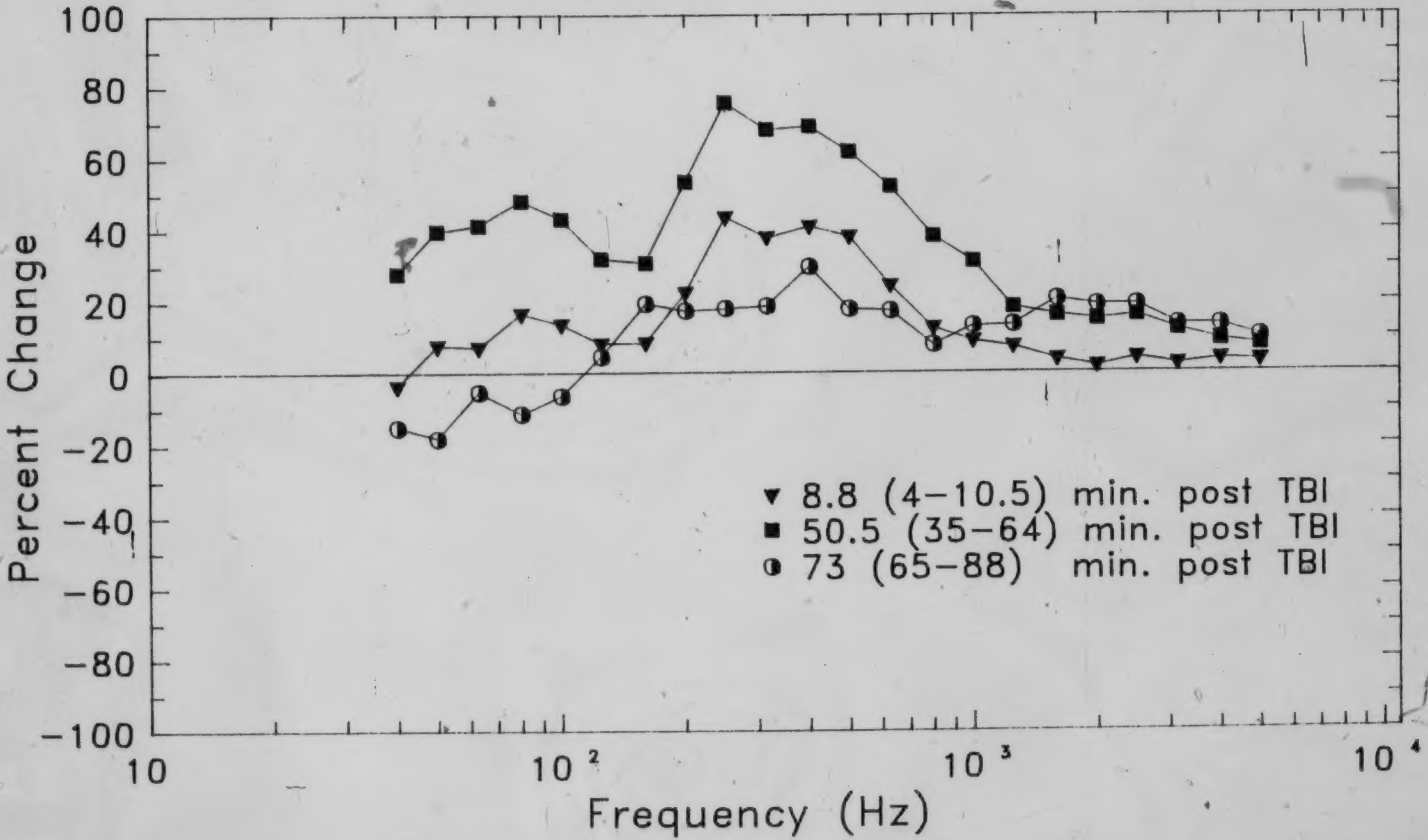


Figure 31. The per cent change in each frequency band of the vagal power spectrum after TBI, for one rabbit less than 100 days old which exhibited the deliberate response. This animal produced power spectra after irradiation which had neural peak voltage amplitudes that were clearly lower than those of the pre-irradiation power spectrum. The per cent change is shown for three different times after irradiation; the figure legend indicates these times. The vertical axis shows the per cent change, going from -100% to +100%, and the horizontal axis is of frequency, scaled logarithmically ($\log_{10}$).

Fig. 31: Power Spectrum After TBI – Deliberate

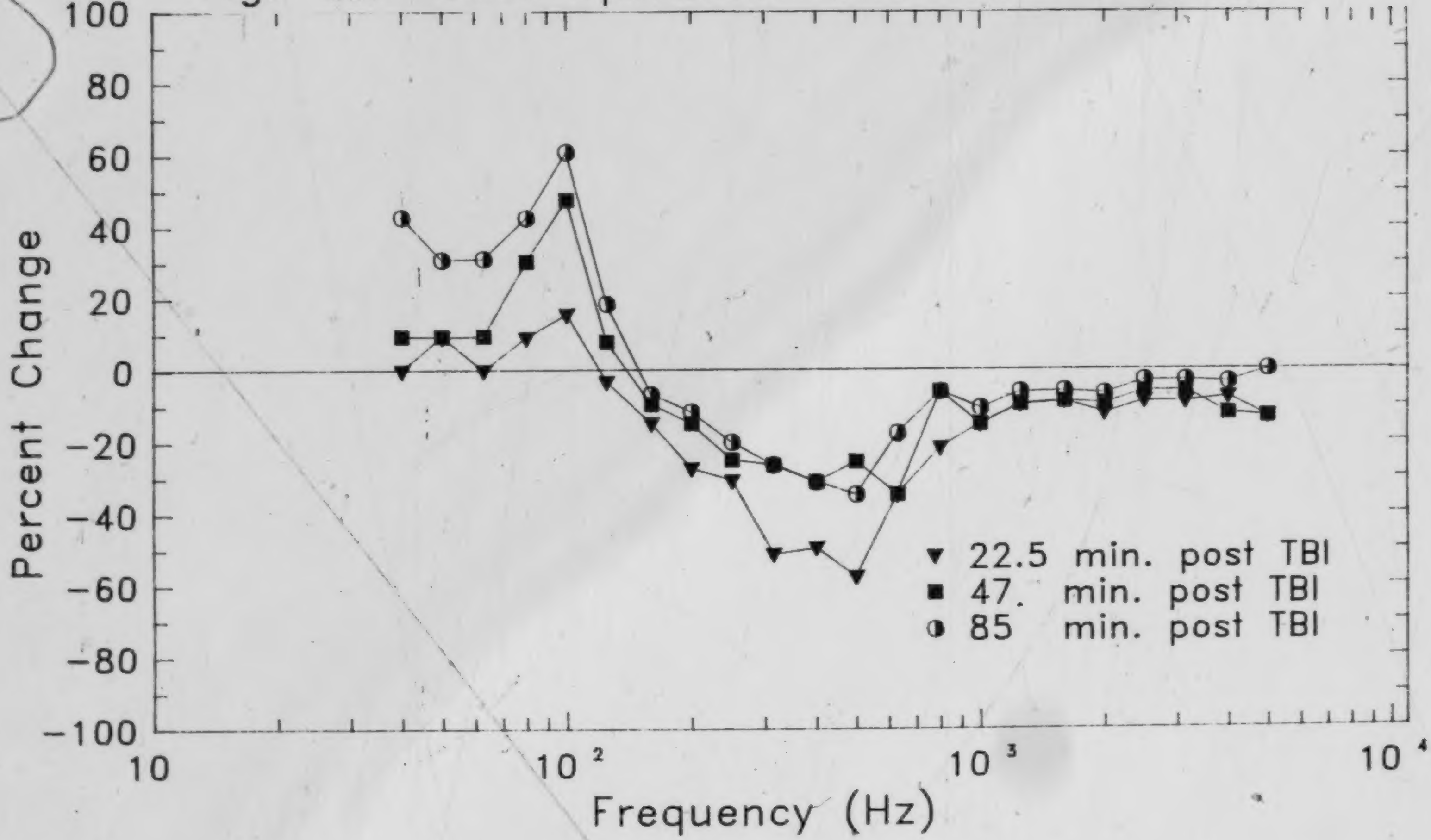


Figure 32. Chart recording of heart rate (top) and cylinder tip temperature (bottom) for a rabbit which was fitted with the vagal cooler. The heart rate scale is from 0 to 500 BPM, and the temperature scale is from -50 C to +50 C. Horizontally, each major division of the chart grid represents one minute of recording time. The arrow indicated "B" on the recording is at 51.5 minutes after the cooling experiment was begun.

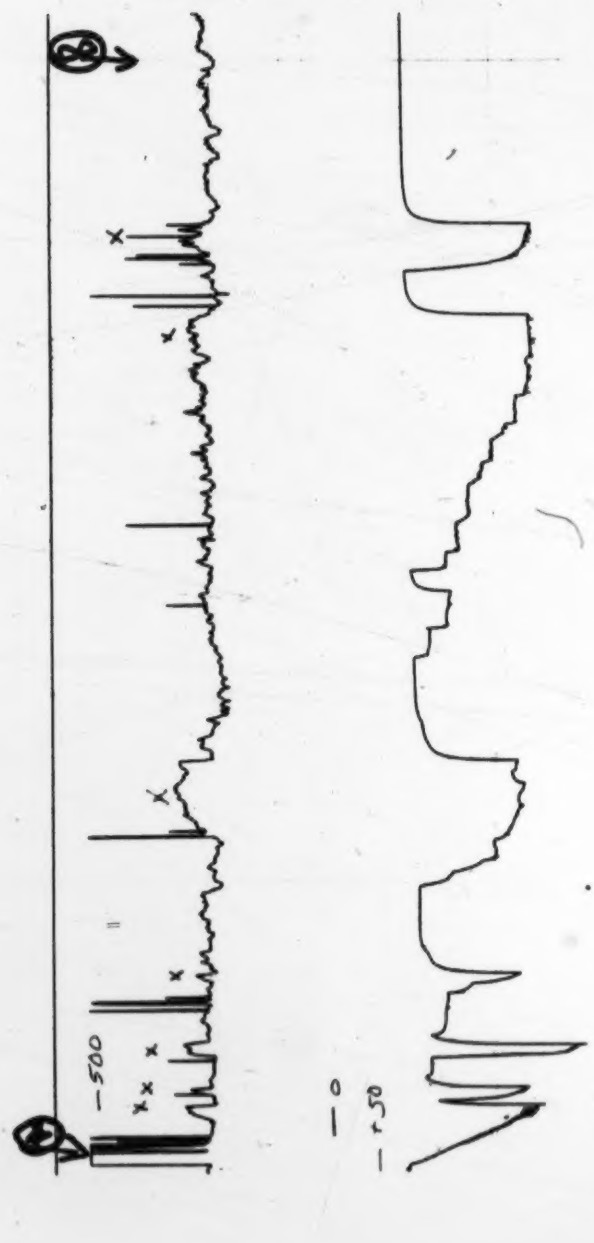


Figure 32.

Figure 33. Reproduction of the chart recording obtained from a 59 day old rabbit which was catheterized in the right ventricle and in the dorsal aorta. The animal experienced ACC after TBI with 1197 cGy. The acute drop began at about 74 minutes after TBI; each major division on the chart grid indicates one minute of recording time. The top tracing is of right ventricular pressure, and the scale is from 0 to 100 mm Hg. The bottom tracing is aortic pressure, and the scale is from 0 to 200 mm Hg. Note that the right ventricular pressure started to rise well before the onset of the acute drop.

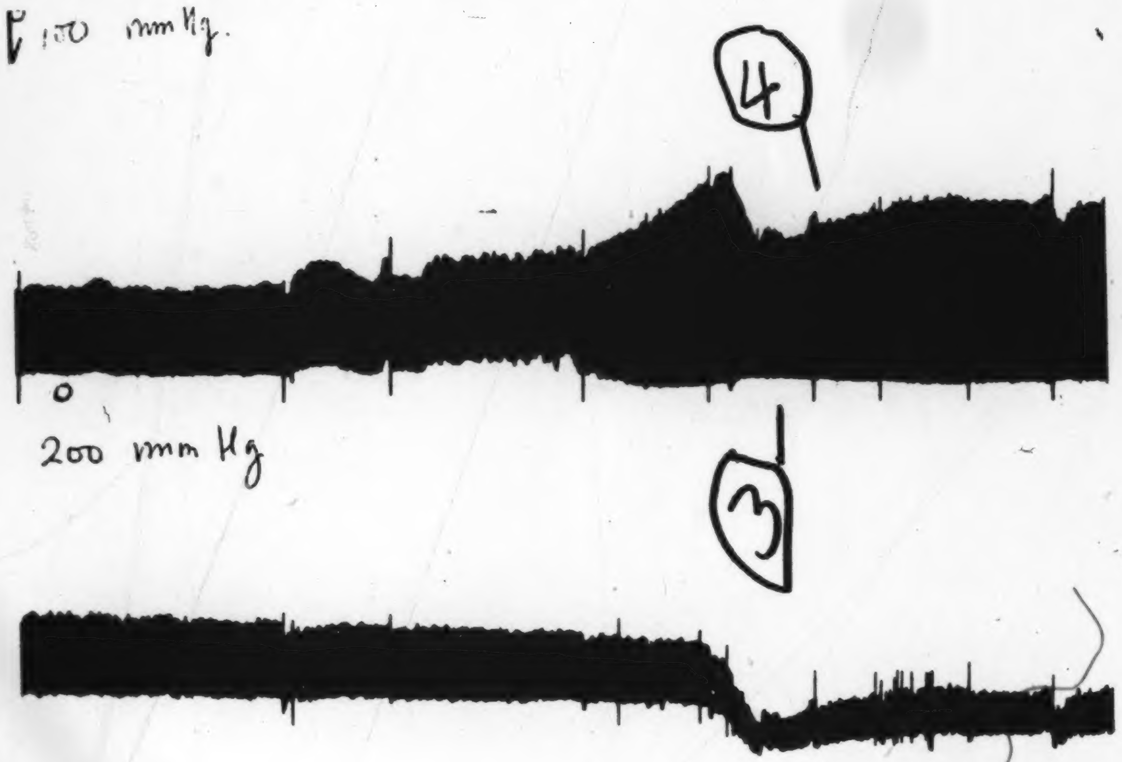


Figure 33. ACC in a rabbit which was catheterized in the right ventricle.

Figure 34. Reproduction of the chart recording obtained from a 53 day rabbit which was catheterized in the right ventricle and in the dorsal aorta. The animal displayed the deliberate response following TBI with 1203 cGy. The top tracing is the right ventricular pressure, and the scale is from 0 to 100 mm Hg. The bottom tracing is the aortic pressure, and the scale is from 0 to 200 mm Hg. The time in minutes from the beginning of irradiation is indicated on the recording just below the aortic pressure tracing.

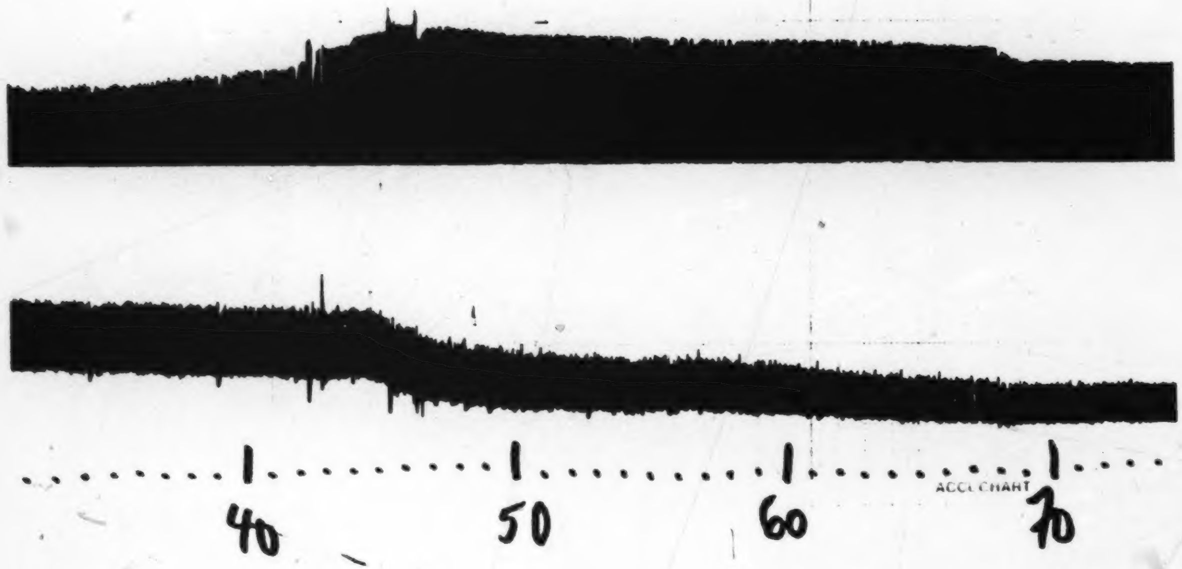


Figure 34. Deliberate hypotension in a rabbit which was catheterized in the right ventricle.

Figure 35. The change in right ventricular pressure and aortic pressure with time after TBI, for three rabbits which displayed ACC. This figure was produced by first digitizing the systolic and diastolic right ventricular and aortic pressures on the chart records for every 0.5 minute mark. Then an IBM PC/AT computer located the time at which peak right ventricular pressure occurred for each rabbit, calculated the average time, and shifted the right ventricular and aortic data for each rabbit so that peak right ventricular pressures for all three fell on the average time. The pressure data for the three rabbits were then added and divided by 3, to yield average systolic and average diastolic pressures for the three animals. These values were then plotted. A 20 minute pre-irradiation pressure record interval was also digitized for each rabbit. The pressures were averaged for the three animals and the averages were plotted to lie between -20 and 0 minutes before TBI. (The digitized values however, were obtained for any 20 minute pre-exposure interval.) The systolic and diastolic points were each connected with a line, and the area between the systolic line and the diastolic line was filled in either with dots or with hatching. The dotted band is the aortic pressure, and the hatched band is the right ventricular pressure. The vertical axis is pressure in mm Hg, going from -10 mm Hg to 200 mm Hg. The horizontal axis shows the time in minutes before and after the beginning of TBI.

Fig. 35: RV and Aortic Pressures — Acute

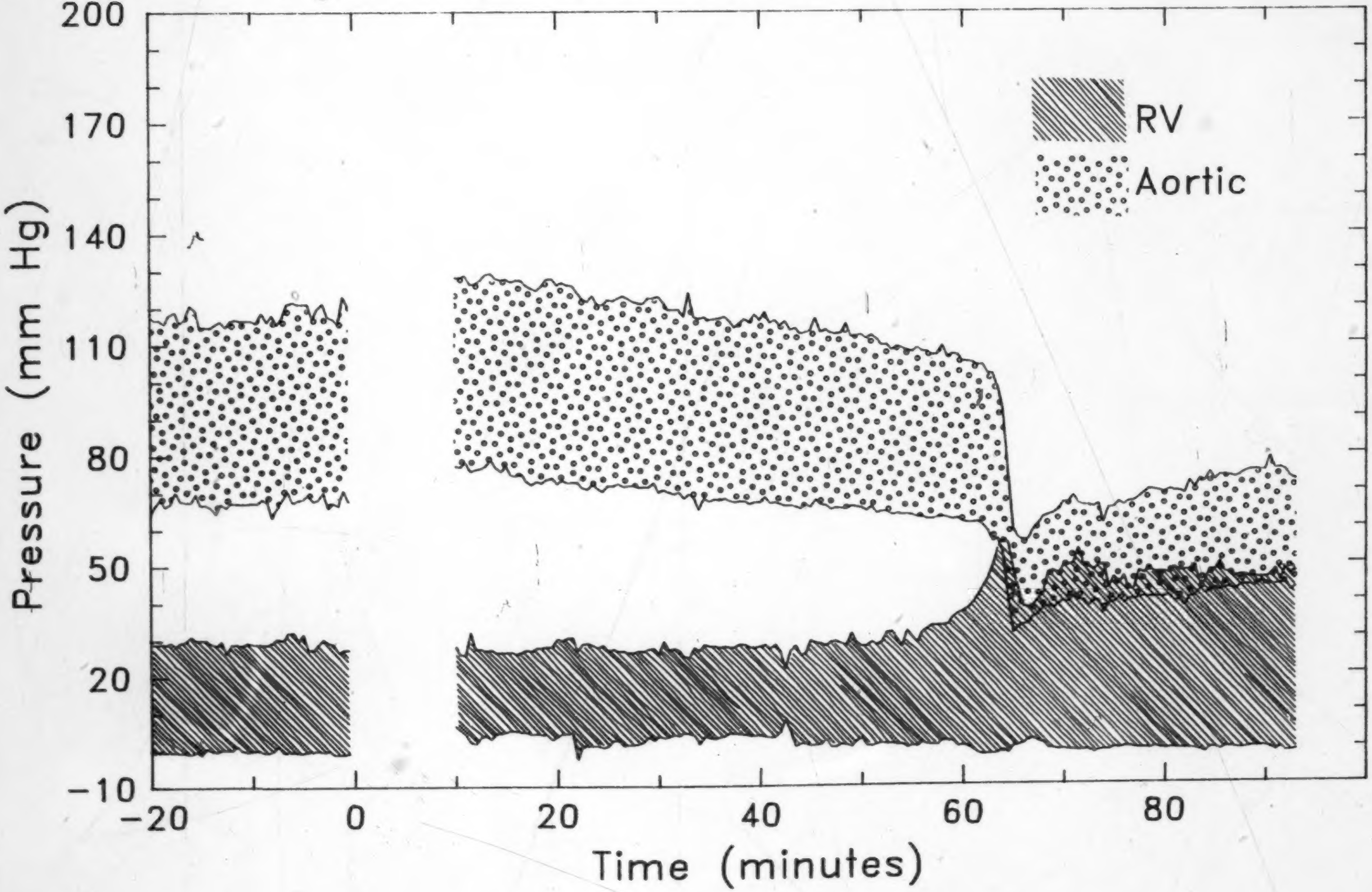


Figure 36. The change in right ventricular pressure and aortic pressure with time after TBI, for one rabbit which displayed ACC. The figure was prepared in the same way as Figure 35, only that no averaging nor shifting of data was done. The rabbit was not grouped with the three acute responders of Figure 35 because its pressure record was erratic, and would have caused Figure 35 to be less decisive. The dotted band represents aortic pressure, and the hatched band constitutes right ventricular pressure. The vertical axis is pressure in mm Hg, going from -10 to 200 mm Hg, and the horizontal axis shows the time in minutes before and after the beginning of TBI.

Fig. 36: RV and Aortic Pressures – Single Acute

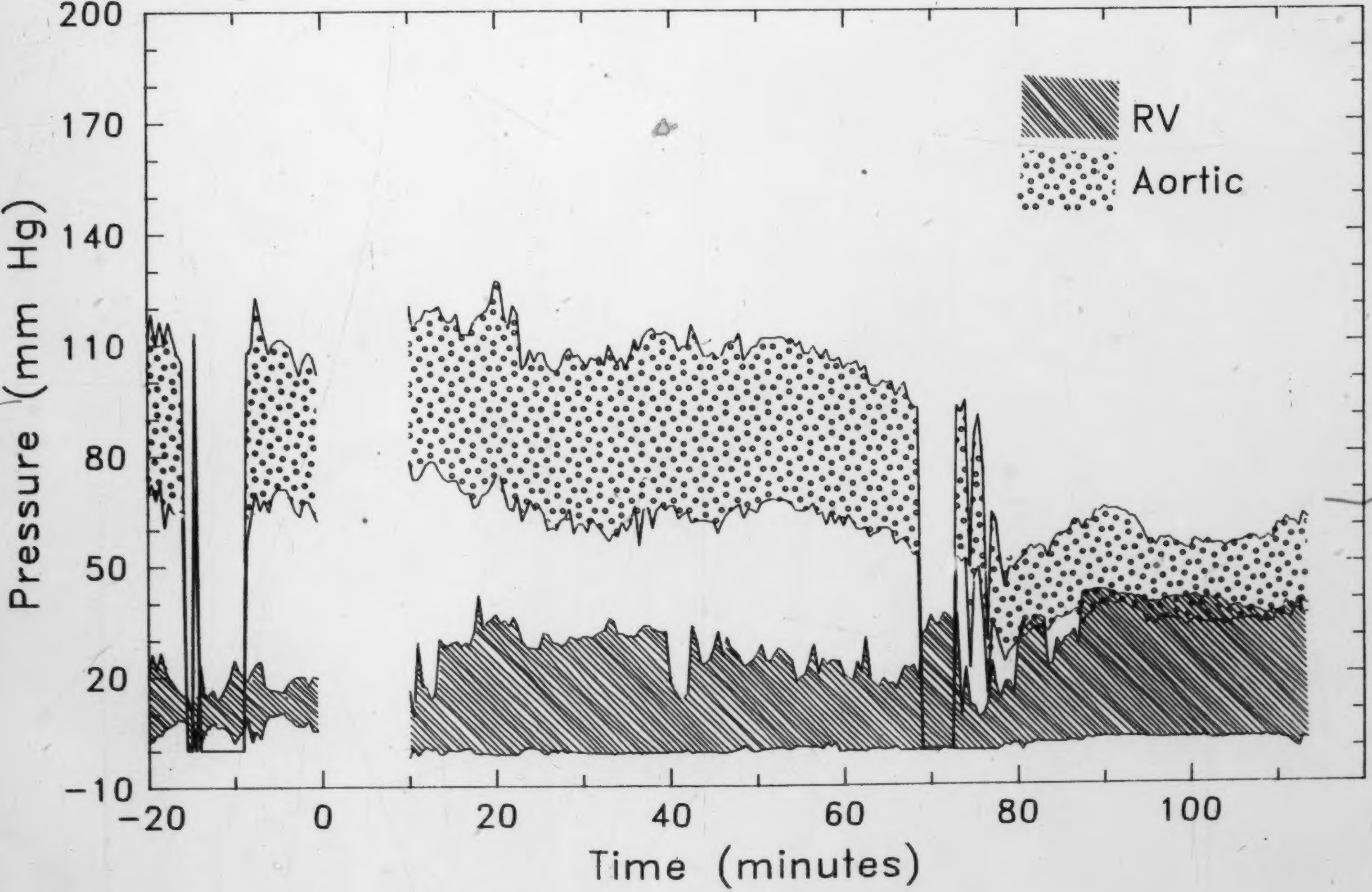


Figure 37. The change in right ventricular pressure and aortic pressure with time after TBI, for two rabbits which displayed steep deliberate declines in aortic pressure. The figure involved averaging and shifting of data, and was produced in the same way as Figure 35. However, the data were not shifted according to peak right ventricular pressure, but rather in relation to the time at which the systolic aortic pressure began to shoulder down steeply. The data were shifted so that the two shoulders matched. The dotted band represents aortic pressure, and the hatched band denotes right ventricular pressure. The vertical axis is pressure in mm Hg, going from -10 to 200 mm Hg. The horizontal axis shows the time in minutes before and after the beginning of TBI.

Fig. 37: RV and Aortic Pressures – Steep Deliberate

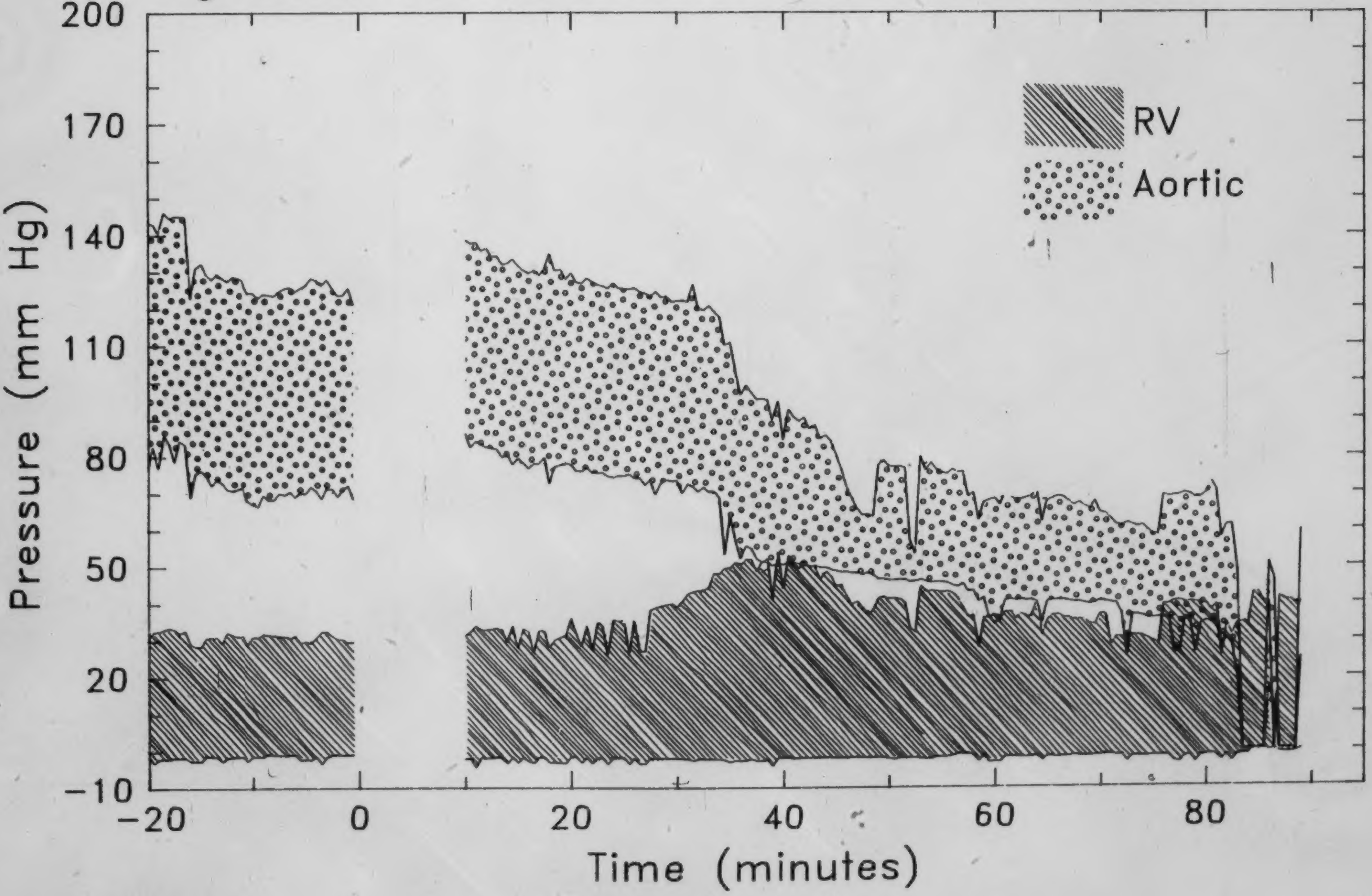


Figure 38. The change in right ventricular pressure and aortic pressure with time after TBI, for two rabbits which displayed relatively shallow deliberate declines in aortic pressure. The pressure data for the two rabbits were added and divided by 2, to yield average systolic and average diastolic pressures. The average values were plotted. No shifting of data was performed. The dotted band represents the aortic pressure, and the hatched band denotes the right ventricular pressure. The vertical axis is pressure in mm Hg, going from -10 to 200 mm Hg. The horizontal axis shows the time in minutes from the beginning of TBI.

Fig. 38: RV and Aortic Pressures – Deliberate

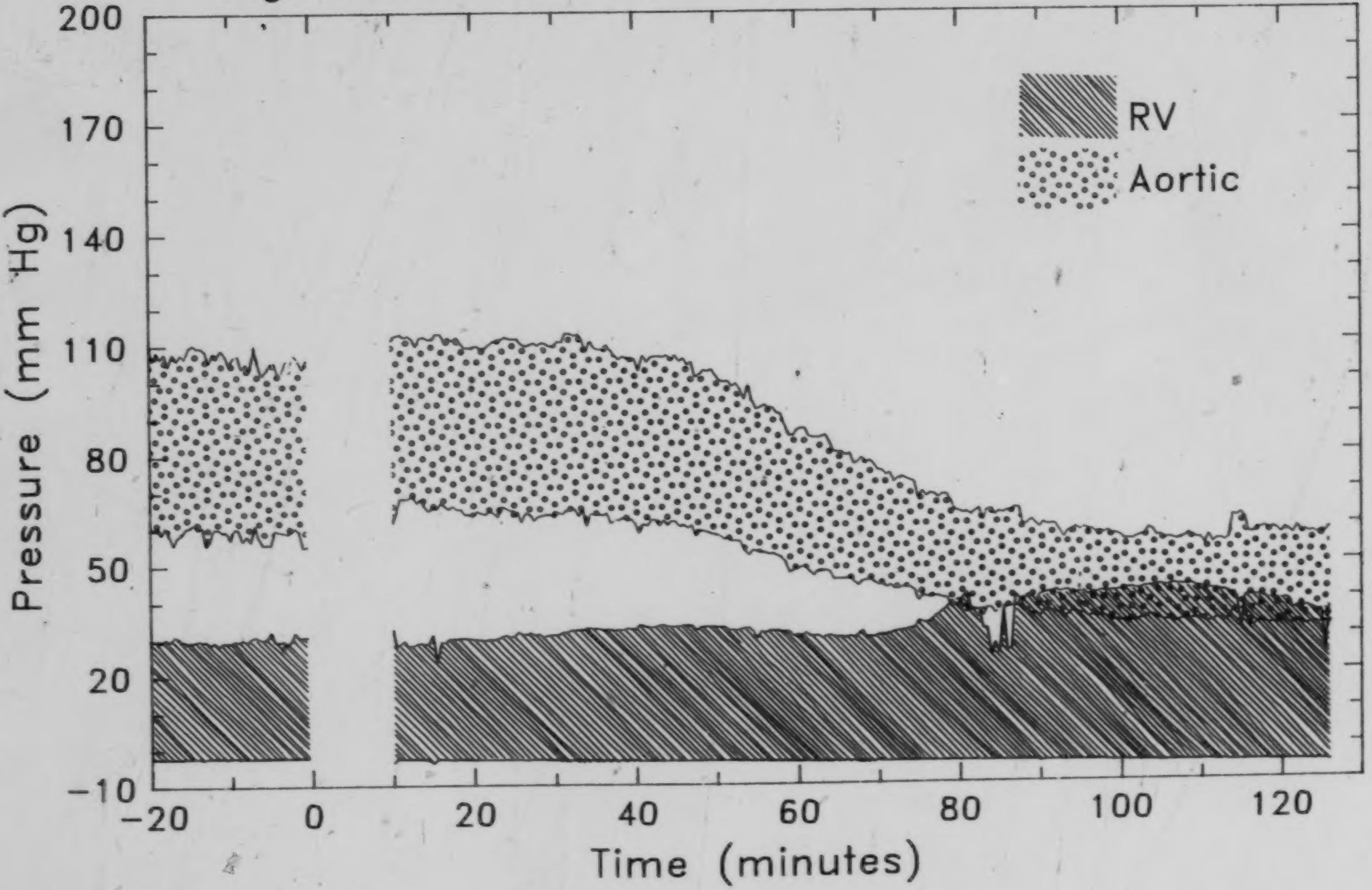


Figure 39. The change in right ventricular pressure and aortic pressure with time after TBI, for two rabbits which displayed deliberate declines in aortic pressure. The two rabbits represented in this figure differ from the two deliberate responders of Figure 38 in that they (rabbits of Fig. 39) experienced a decline in right ventricular pressure, from the pre-exposure level, after TBI. The right ventricular pressure for the rabbits of Figure 38 increased following TBI. For this figure (39), the pressure data for the two rabbits was added and divided by 2, to yield average systolic and diastolic pressures. The average values were plotted, without any shifting of data. The dotted band represents aortic pressure, and the hatched band constitutes right ventricular pressure. The vertical axis indicates pressure in mm Hg, going from -10 to 200 mm Hg. The horizontal axis shows the time in minutes from the beginning of TBI.

Fig. 39: RV and Aortic Pressures – Shallow

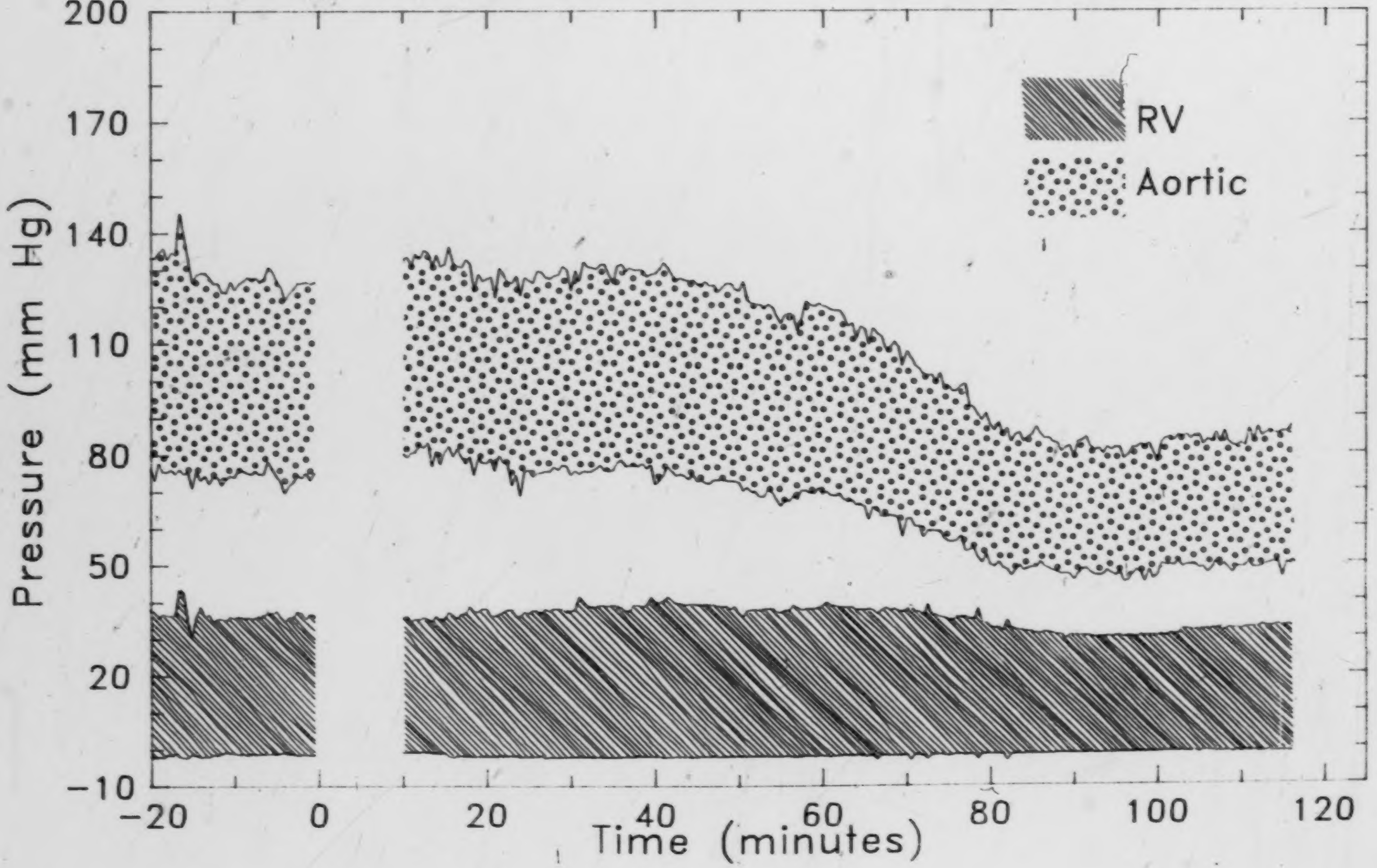


Figure 40. The change in mean arterial pressure (MAP) and in the ratio of mean right ventricular pressure (MRVP) to MAP, with time after TBI, for three rabbits which displayed ACC. This figure was produced by first having an IBM PC/AT computer scan the files containing digitized right ventricular pressures, for the time at which the peak pressure occurred for each of the three rabbits. The computer then calculated the average peak time for the group, and shifted the right ventricular and aortic pressure data for each animal so that the peak right ventricular pressures fell on the calculated average time. Then MAP and MRVP were calculated using the formula; 1/3 systolic + 2/3 diastolic. After the means were determined, MRVP was divided by MAP, and MAP and the quotient (ratio) of MRVP/MAP were each added and divided by 3, to produce average MAP and average ratio of MRVP/MAP for the three rabbits as a group. The average MAP was plotted on the figure (heavy solid line) as was the average ratio MRVP/MAP (light solid line). MAP and MRVP were calculated for each rabbit for a 20 minute pre-irradiation time interval, and the ratio MRVP/MAP was determined. MAP and the ratio MRVP/MAP were averaged for the group of rabbits, and plotted on the figure to lie between -20 and 0 minutes prior to TBI. The pre-irradiation MAP and ratio of MRVP/MAP were prepared in this way for Figures 40 to 44, with the exception of Figure 41, which did not involve any averaging between rabbits as only one animal is presented. The left vertical axis is pressure in mm Hg, the horizontal axis shows the time in minutes before and after the beginning of TBI, and the right vertical axis indicates the ratio MRVP/MAP.

Fig. 40: Mean Aortic vs. MRVP/MAP – Acutes

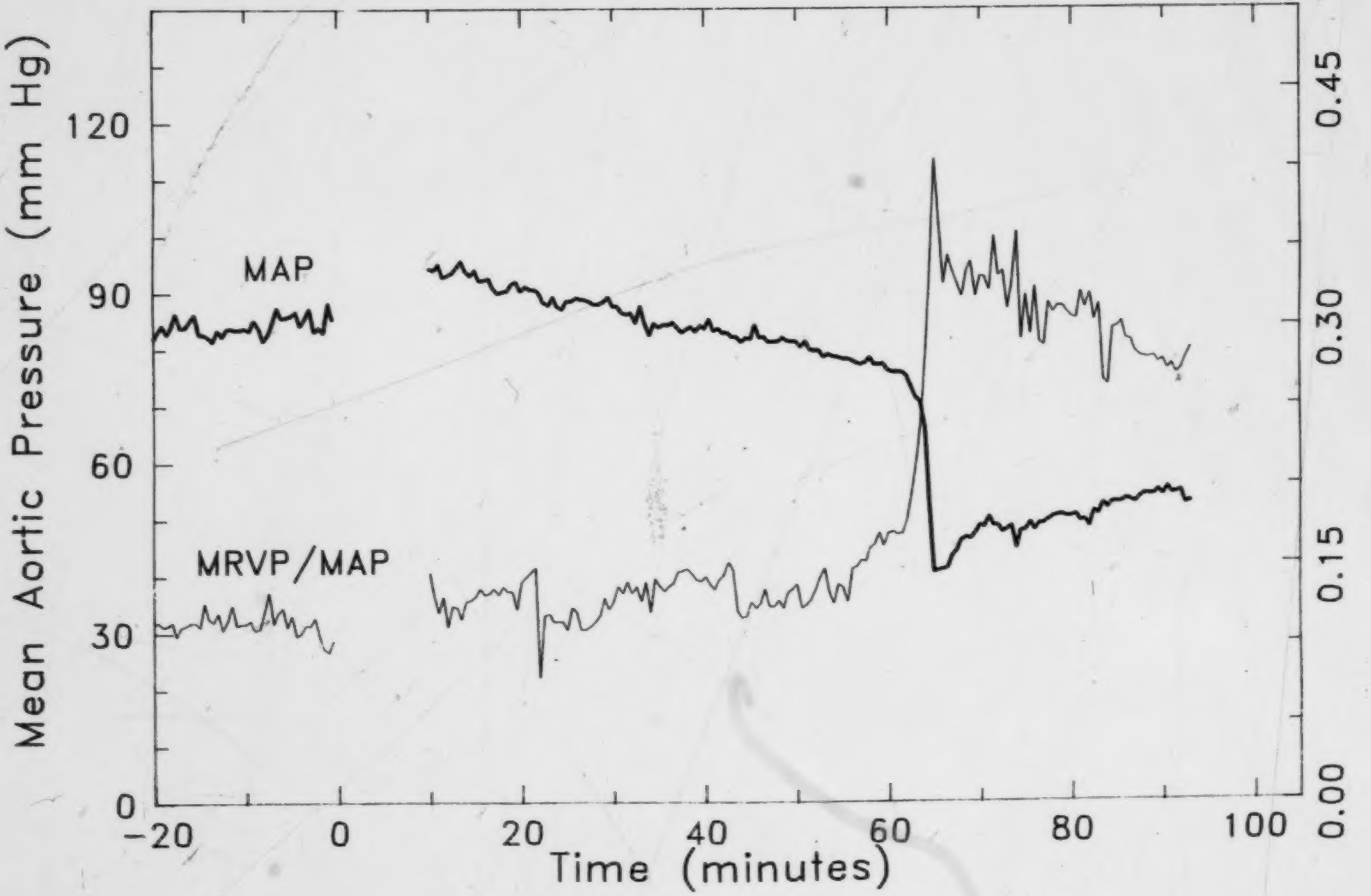


Figure 41. The change in mean arterial pressure (MAP) and in the ratio of mean right ventricular pressure (MRVP) to MAP, with time after TBI, for one rabbit which displayed ACC. This animal was not grouped with the acute responders presented in Figure 40 because its pressure record was erratic, and if this data were included, Figure 40 would have less sharply portrayed how right ventricular changes during ACC. For the preparation of this figure (41), MAP, MRVP, and the ratio MRVP/MAP were calculated, and MAP (heavy line) and the ratio MRVP/MAP (light line) were plotted on the graph. The left vertical axis is pressure in mm Hg, the horizontal axis shows the time in minutes after and before the beginning of TBI, and the right vertical axis indicates the ratio MRVP/MAP.

Fig. 41: Mean Aortic vs. MRVP/MAP – Acute

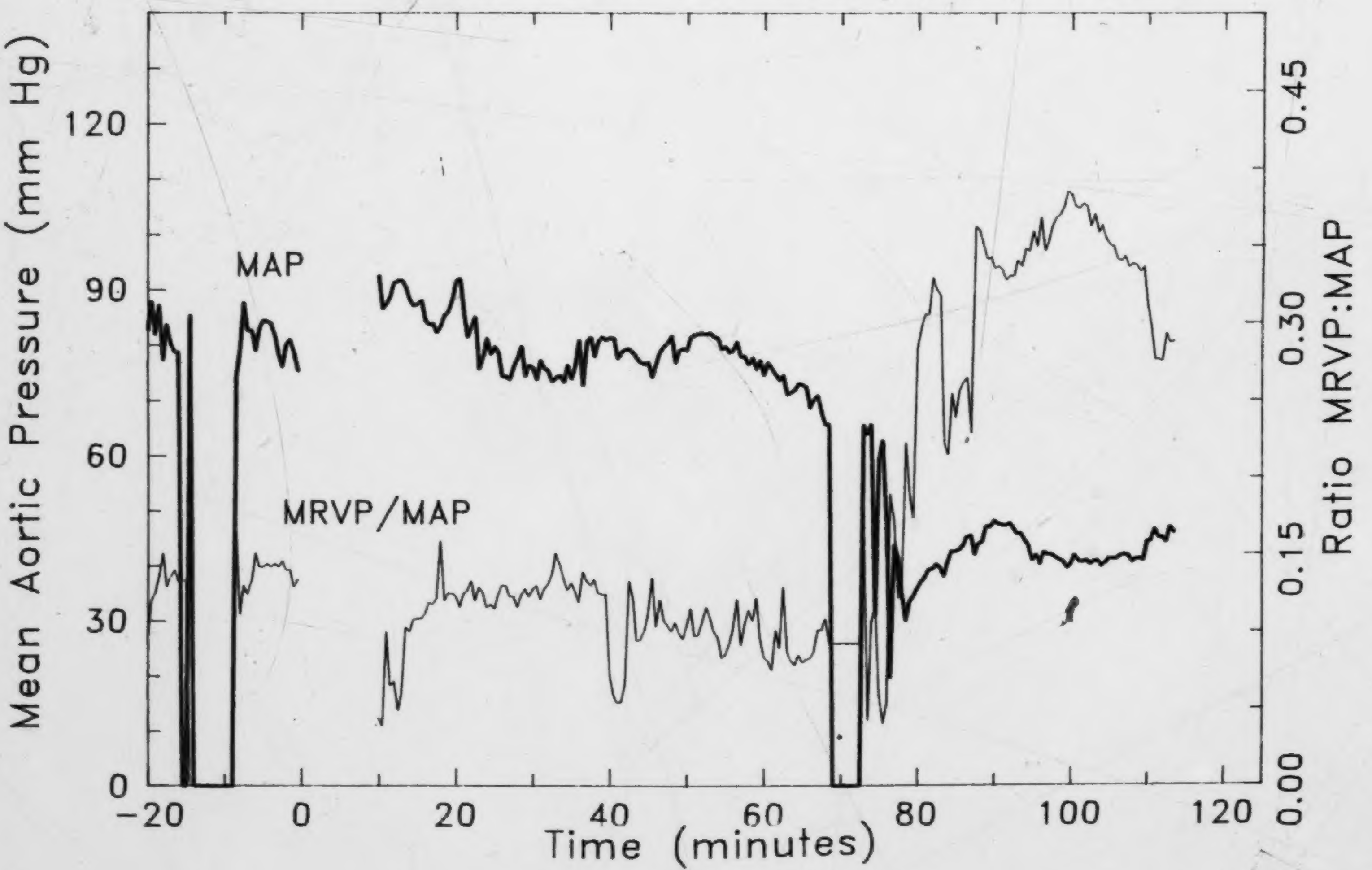


Figure 42. The change in mean arterial pressure (MAP) and in the ratio of mean right ventricular pressure (MRVP) to MAP, with time after TBI, for two rabbits which displayed a steep deliberate decline in aortic pressure. The pressure data was shifted so that the time at which the aortic pressure began to shoulder down steeply matched between the data files for the two rabbits. Then MAP, MRVP, and the ratio MRVP MAP were calculated. MAP and the ratio MRVP/MAP were each averaged between the two rabbits. The average MAP (heavy line) and the average ratio of MRVP/MAP (light line) were plotted. The left vertical axis is pressure in mm Hg, the horizontal axis shows the time in minutes before and after the beginning of TBI, and the right vertical axis indicates the ratio MRVP/MAP.

Fig. 42: Mean Aortic vs. MRVP/MAP – Steep

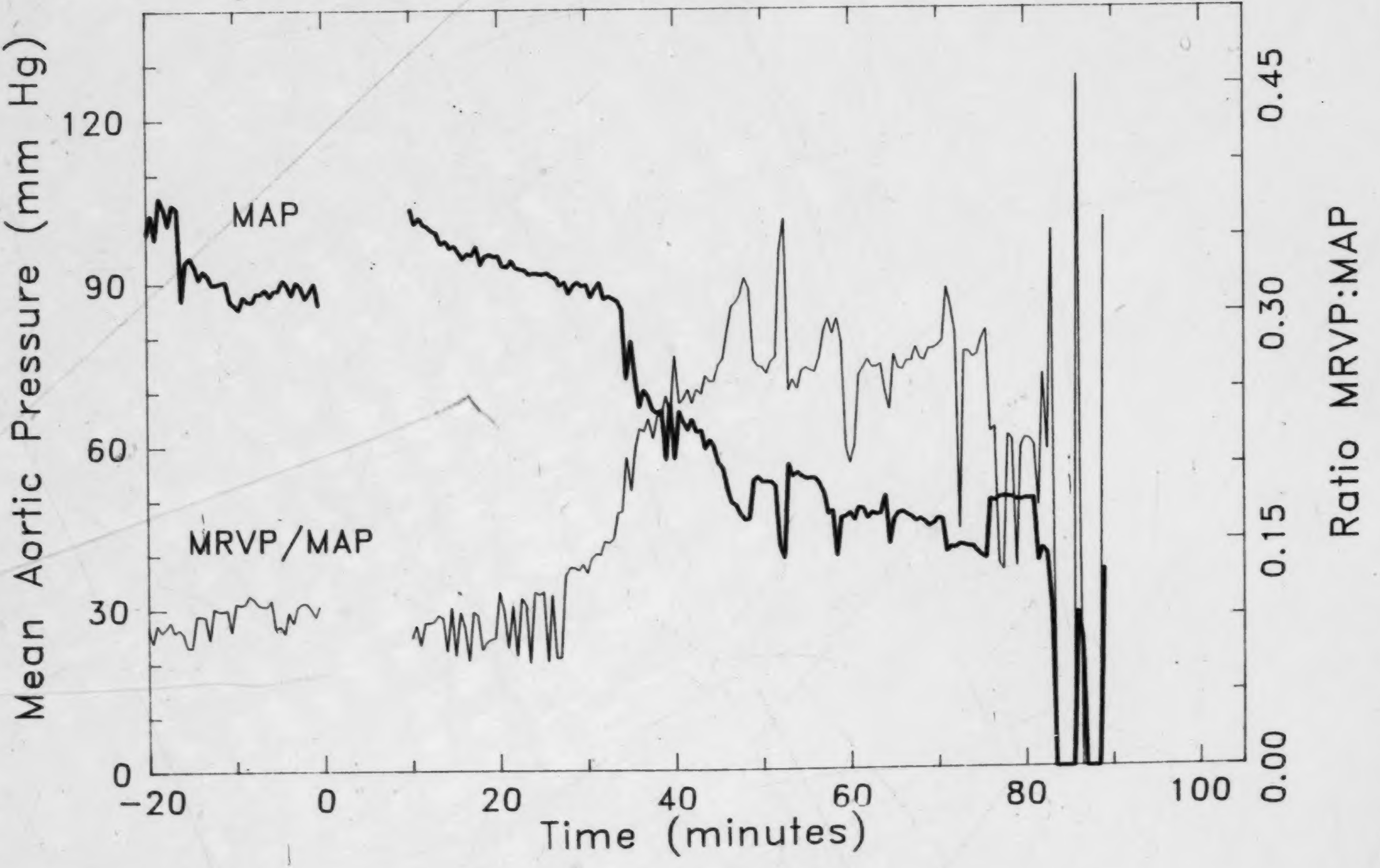


Figure 43. The change in mean arterial pressure (MAP) and in the ratio of mean right ventricular pressure (MRVP) to MAP, with time after TBI, for two rabbits which showed a relatively shallow deliberate decline in aortic pressure. MAP, MRVP, and the ratio MRVP/MAP were calculated, and MAP and the ratio MRVP/MAP were each averaged between the two rabbits. The average MAP (heavy line) and the average ratio of MRVP/MAP (light line) were plotted. The left vertical axis is pressure in mm Hg, the horizontal axis shows the time in minutes before and after the beginning of TBI, and the left vertical axis indicates the ratio MRVP/MAP.

Fig. 43: Mean Aortic vs. MRVP/MAP – Deliberates

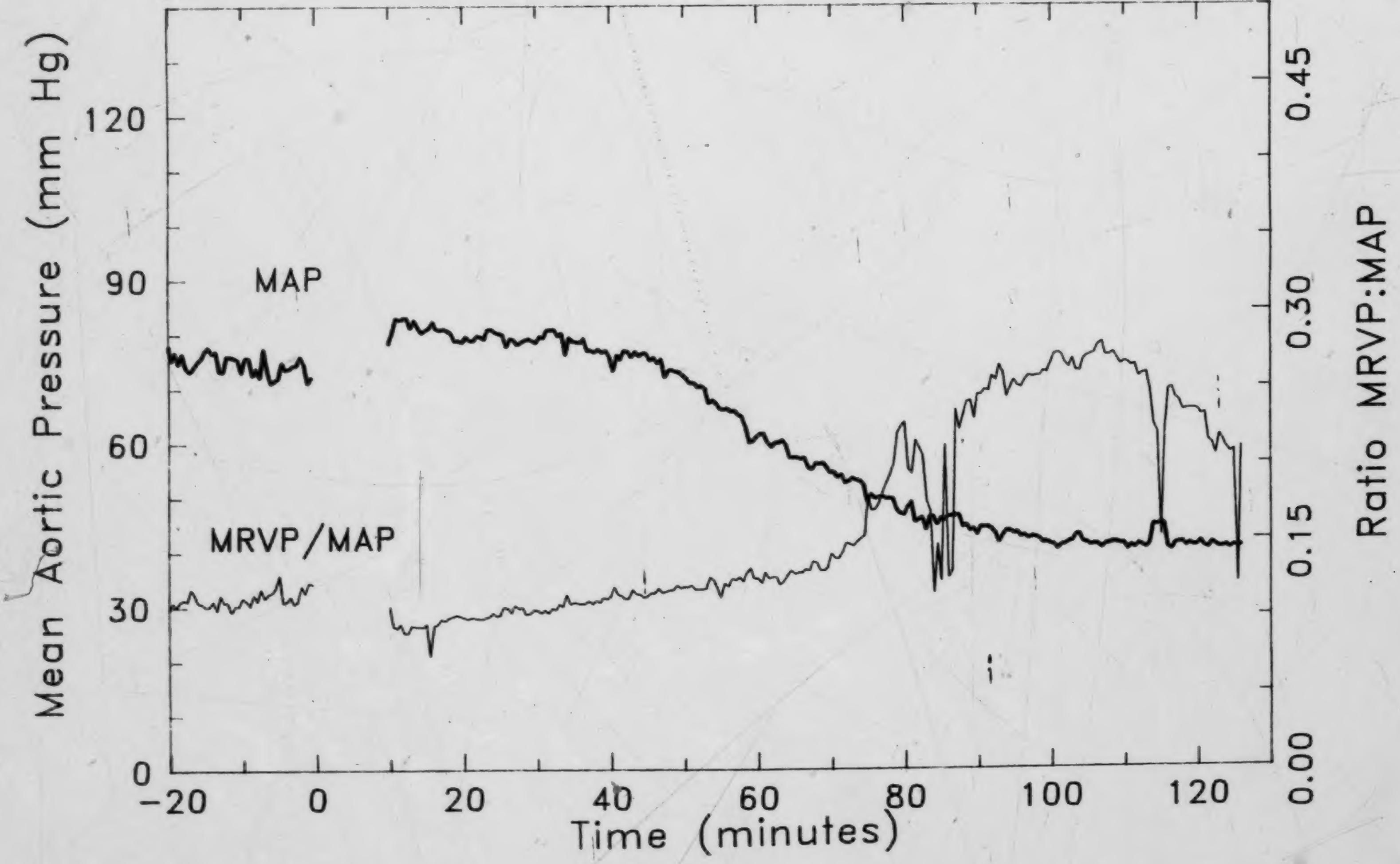


Figure 44. The change in mean arterial pressure (MAP) and in the ratio of mean right ventricular pressure (MRVP) to MAP, with time after TBI, for two rabbits which showed a relatively shallow deliberate decline in aortic pressure, and which displayed lower right ventricular pressure after TBI than prior to TBI. MAP, MRVP, and the ratio MRVP/MAP were calculated, and MAP and the ratio MRVP/MAP were each averaged between the two rabbits. The average MAP (heavy line) and the average ratio of MRVP/MAP (light line) were plotted. The left vertical axis is pressure in mm Hg, the horizontal axis shows the time in minutes before and after the beginning of TBI, and the right vertical axis indicates the ratio MRVP/MAP. Note that the ratio MRVP/MAP rose steadily after TBI.

Fig. 44: Mean Aortic vs. MRVP/MAP – Shallow

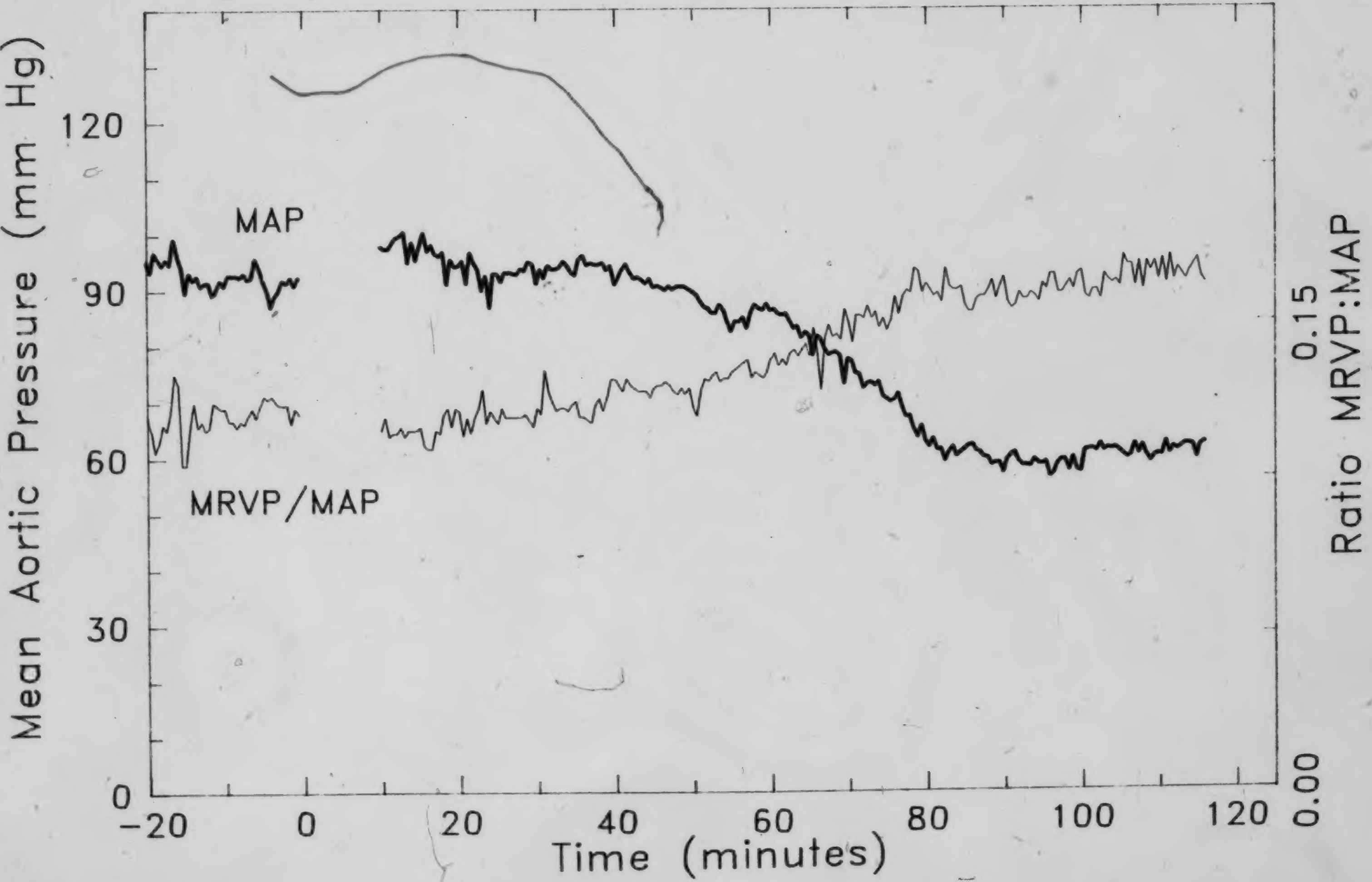
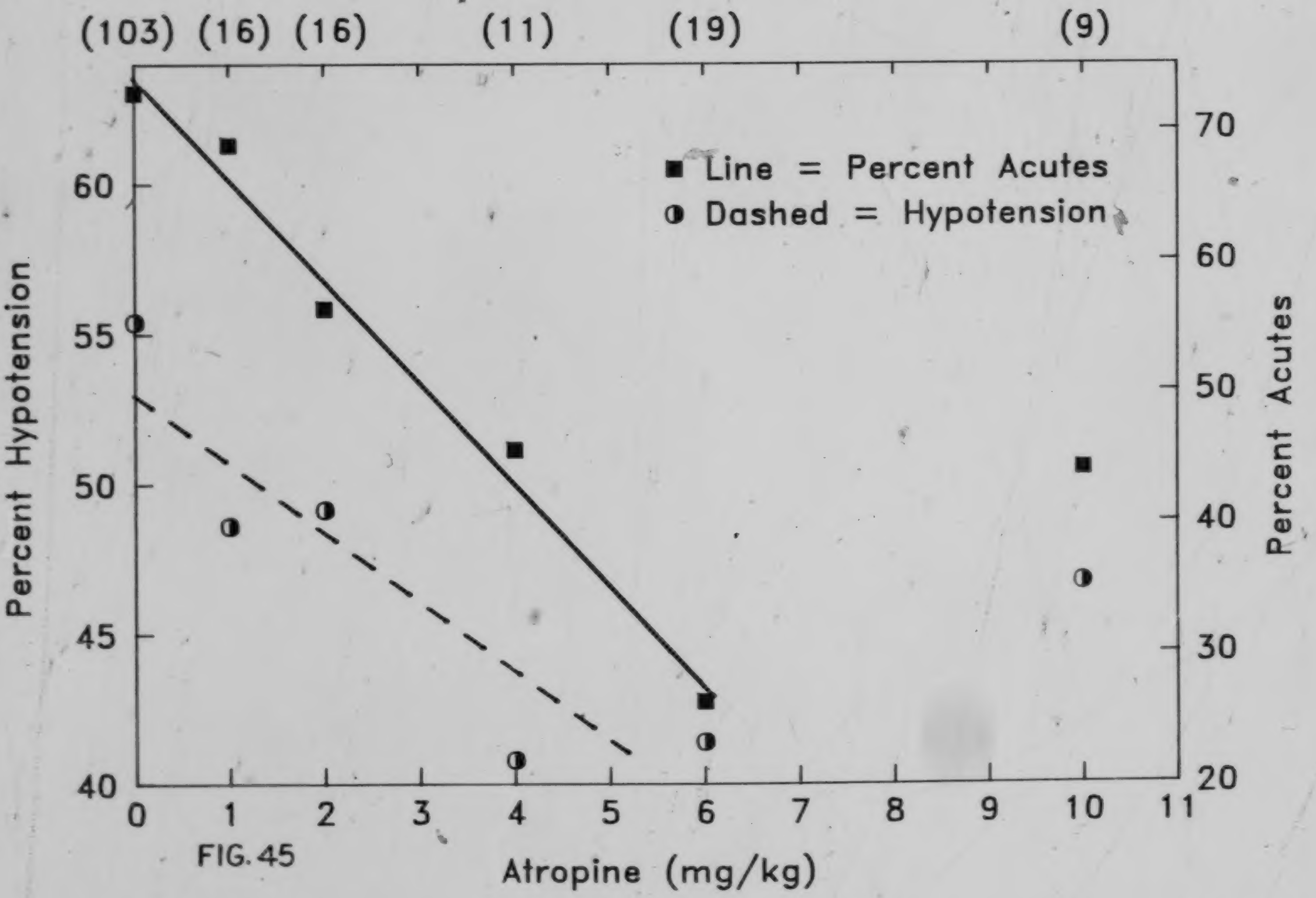


Figure 45. Graph showing how the depth of radiation hypotension and the frequency of ACC vary according to the dose of atropine given. The results using atropine methyl bromide, atropine sulphate, and atropine methyl nitrate were pooled to construct this graph. Also, the results of tests in which the drug was given before TBI were combined with those obtained when the atropine was injected after exposure. The circles indicate the depth of hypotension in per cent, calculated as the per cent decline in mean aortic pressure, for all the rabbits in various dose groups. For each dose, the extent of hypotension for acute and deliberate responders combined, is shown. The dashed line is the regression. The square symbols indicate the proportion of rabbits out of the total number tested in each dose group, which showed ACC. The solid line is the regression. The left vertical axis is the per cent hypotension, going from 40% to 64%. The bottom horizontal axis indicates the dose of atropine in mg/kg, and the top horizontal axis indicates the total number of rabbits tested with each dose. The right vertical axis shows the proportion which experienced ACC, in terms of per cent, out of the total number of animals tested. The results with 10 mg/kg were not included in the regression calculations.


(103)
(16)
(16)
(11)
(19)
(9)
■ Line = Percent Acutes
◐ Dashed = Hypotension
Percent Hypotension
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45
50
55
60
Percent Acutes
20
30
40
50
60
70
0
1
2
3
4
5
6
7
8
9
10
11
Atropine (mg/kg)

FIG. 45

Figure 46. Chart recording for two rabbits which were injected with 1 mg of endotoxin. The two lower tracings are aortic blood pressure, and the scale for each is 0 to 200 mm Hg. The time in minutes after injection is indicated for each pressure recording at the bottom of their respective chart grids. The top tracing is of ear temperature, and the scale is 24 to 40 degrees C. The temperature tracing was taken from the rabbit which produced the lowest blood pressure tracing.

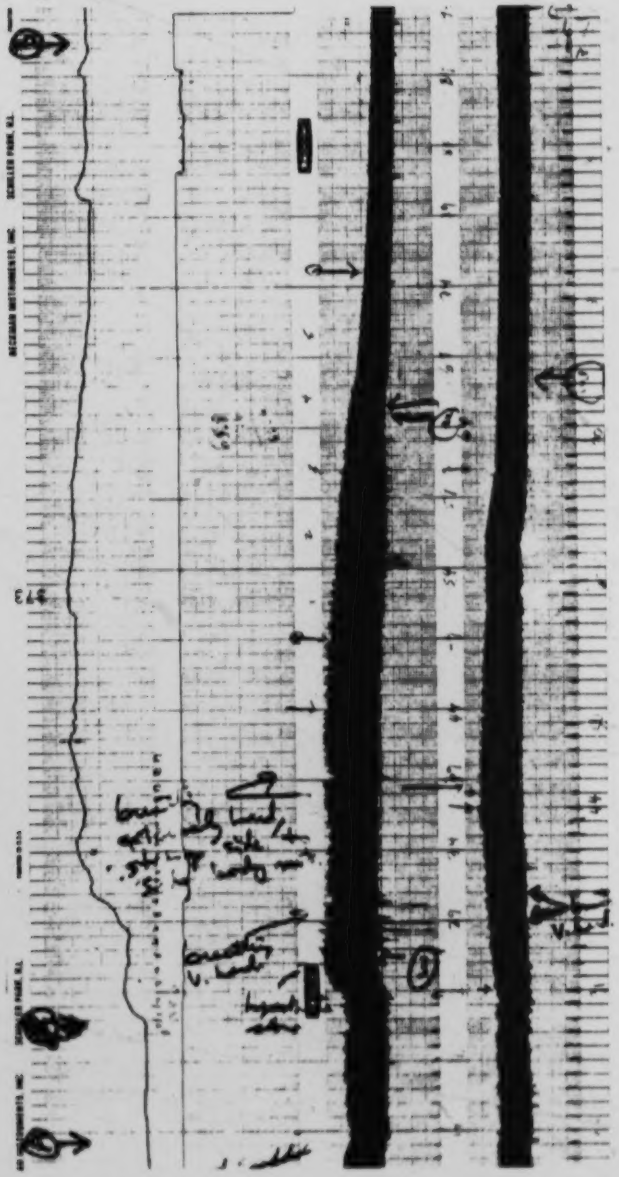

Figure 46. Blood pressure records for two rabbits injected with endotoxin.

Figure 47. Reproduction of the chart recording obtained for a 54 day old rabbit which was bilaterally vagotomized, and which was subjected to heart irradiation with 1200 cGy. The top tracing is the aortic blood pressure, and the vertical scale is from 0 to 200 mm Hg. The time scale for the chart is indicated in minutes from the beginning of irradiation, just below the pressure tracing. The middle tracing is of ear temperature, and the scale is from 22 to 40 degrees C. The bottom tracing is the heart rate, and the scale is from 0 to 500 BPM. Note the rise in blood pressure and in ear temperature at about 75 minutes after irradiation.

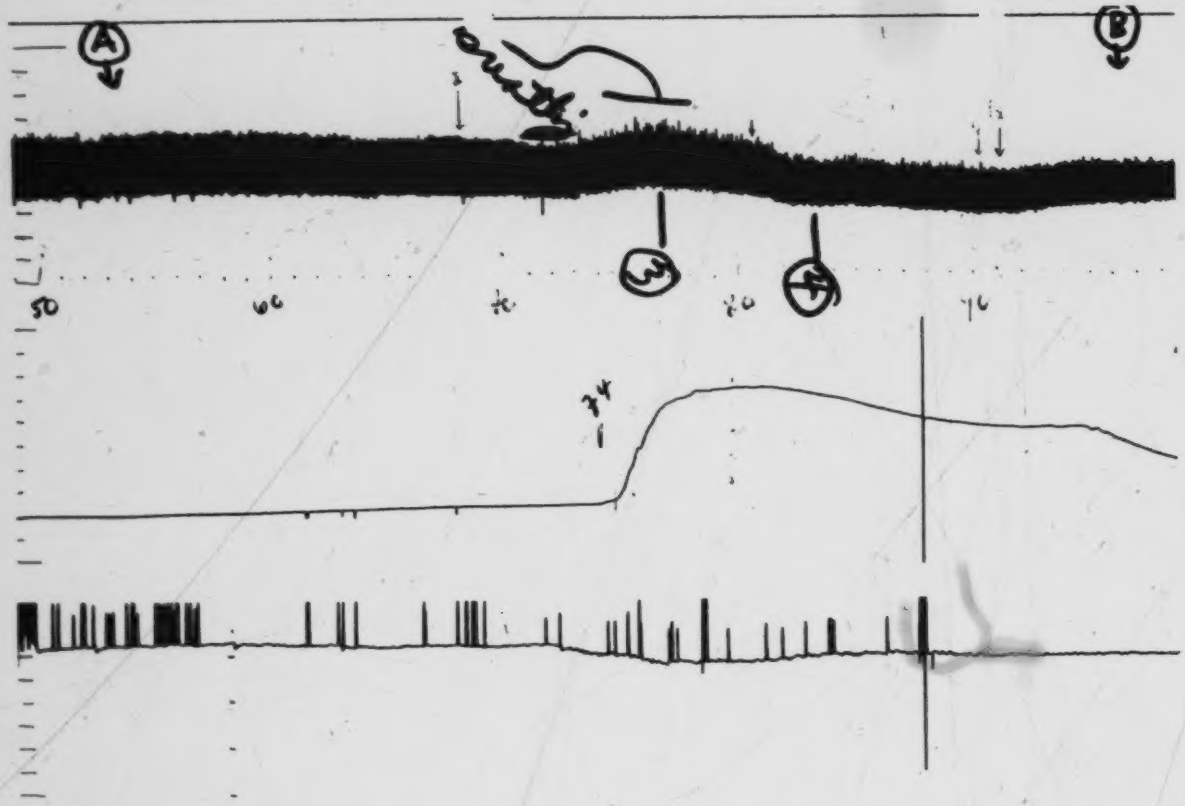

Figure 47. Chart recording for a bilaterally vagotomized rabbit which was subjected to heart irradiation.

Figure 48. Reproduction of the chart recording obtained for a 60 day old rabbit which was bilaterally vagotomized, and subjected to heart irradiation with 1200 cGy. The top tracing is aortic blood pressure, and the scale is from 0 to 200 mm Hg. The chart time scale is indicated in minutes from the beginning of irradiation, just below the pressure tracing. The bottom tracing is ear temperature, and the scale is from 22 to 40 degrees C. Note the rise in the blood pressure and the ear temperature between 80 and 90 minutes after irradiation.

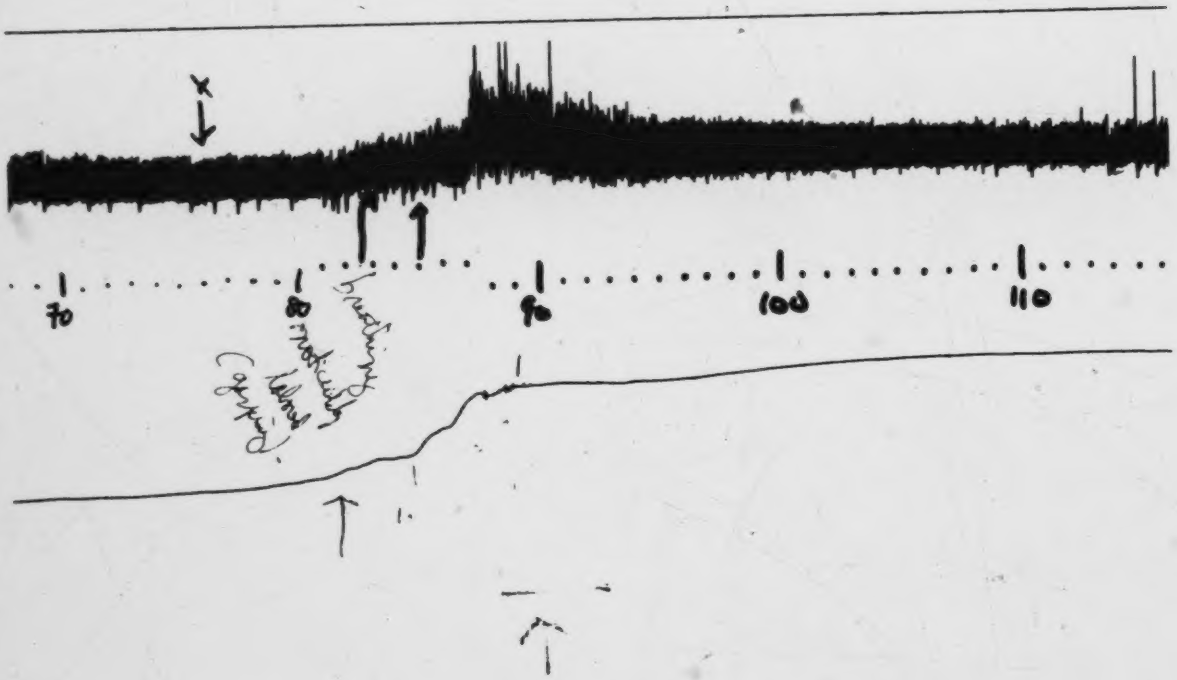

Figure 48. Chart recording for a bilaterally vagotomized rabbit which was subjected to heart irradiation.

Figure 49. The response of a Gould P23 ID pressure transducer to a sudden step-up in pressure, when connected to the source with polyethylene PE-10 tubing. The horizontal scale is 40 mS per major division. The tracing has been darkened by hand with a marking pen.

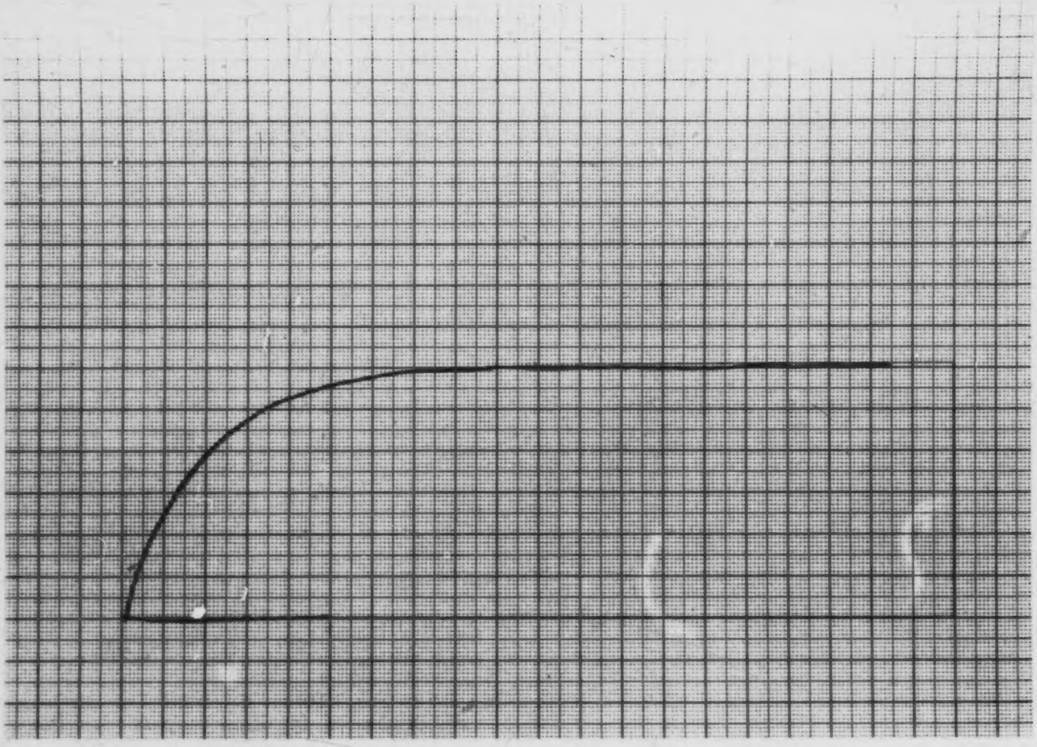

Figure 49.

Figure 50. The response of a Gould P23 ID pressure transducer to a sudden step-up in pressure, when connected to the source with polyethylene PE-190 tubing. The horizontal scale is 4 mS per major division. The tracing was darkened by hand using a marking pen.

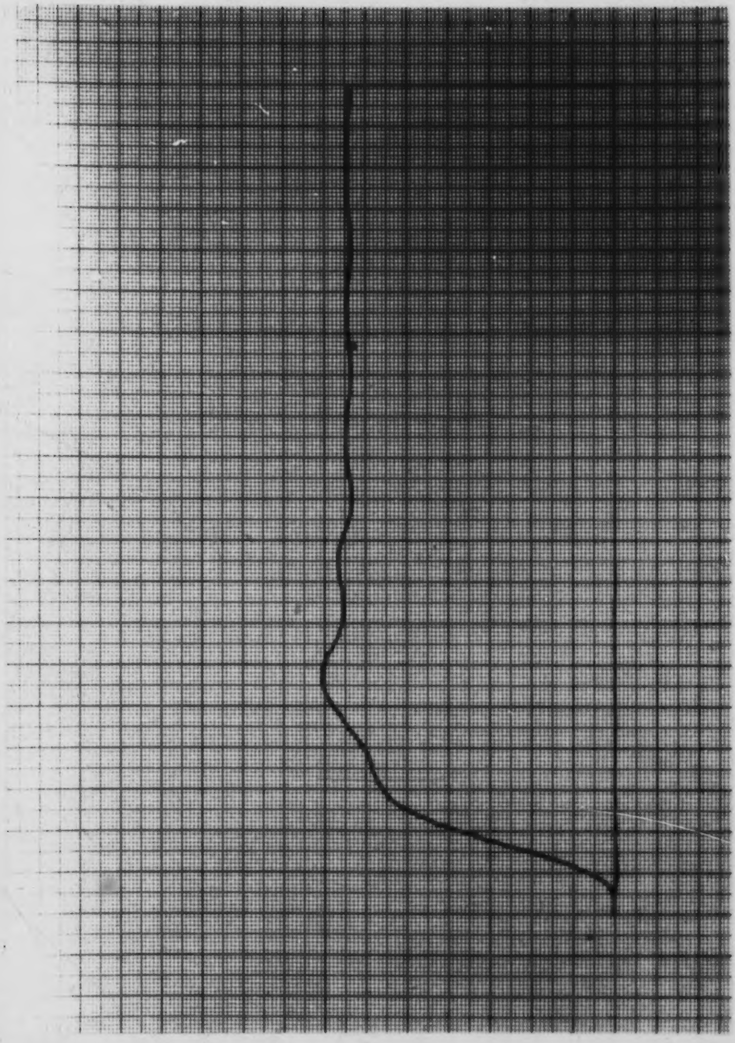

Figure 50.

Figure 51. The response of a Gould P23 ID pressure transducer connected to the pressure wave generator with polyethylene PE-10 tubing. The continuous sine wave was generated to mimic blood pressure waves developing at a frequency of 360 per minute. The tracing was darkened by hand using a marking pen.

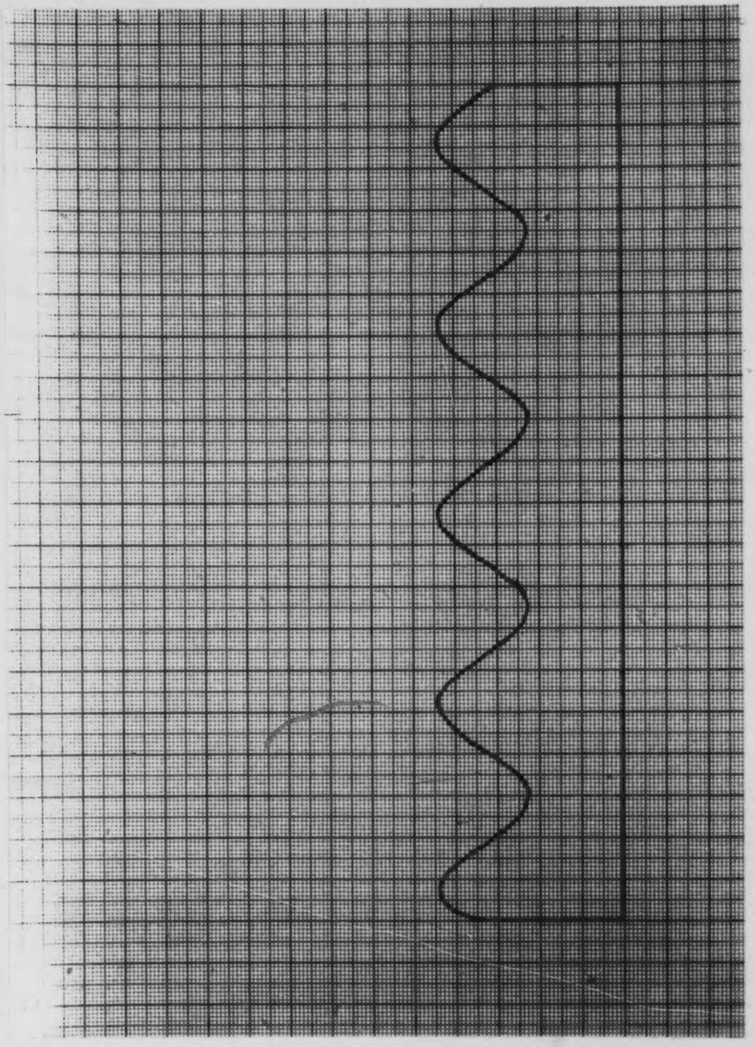

Figure 51.

Figure 52. The response of a Gould P23 ID pressure transducer connected to the pressure wave generator with polyethylene PE-190 tubing. The continuous sine wave was generated to mimic blood pressure waves developing at a frequency of 360 per minute. Compare the amplitude of this tracing with that of the previous figure. The tracing was darkened by hand using a marking pen.

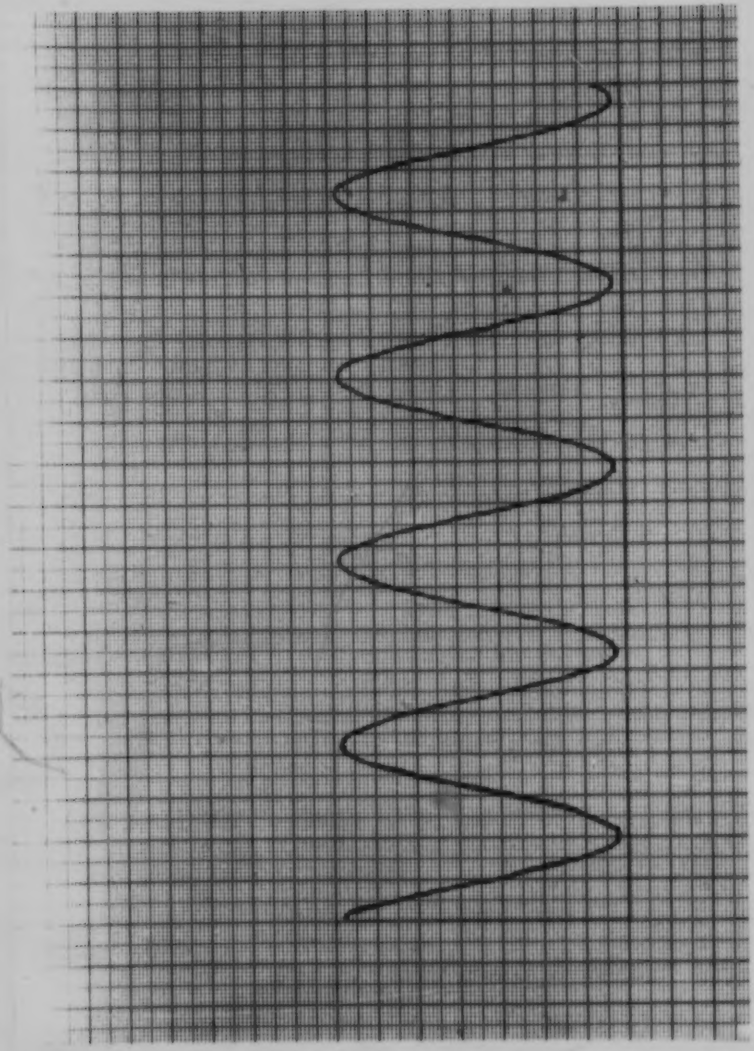

Figure 52.

Figure 53. Reproduction of the chart recording obtained from a 66 day old rabbit which had the arterial pressure recorded using both the cannula and the tail cuff methods simultaneously. This portion of the chart recording was produced prior to TBI. Following irradiation, the cuff method did not work properly. The top tracing is the cuff derived pressure, and the scale is from 0 to 200 mm Hg. The bottom tracing is the cannula derived pressure, and the scale is from 0 to 200 mm Hg.

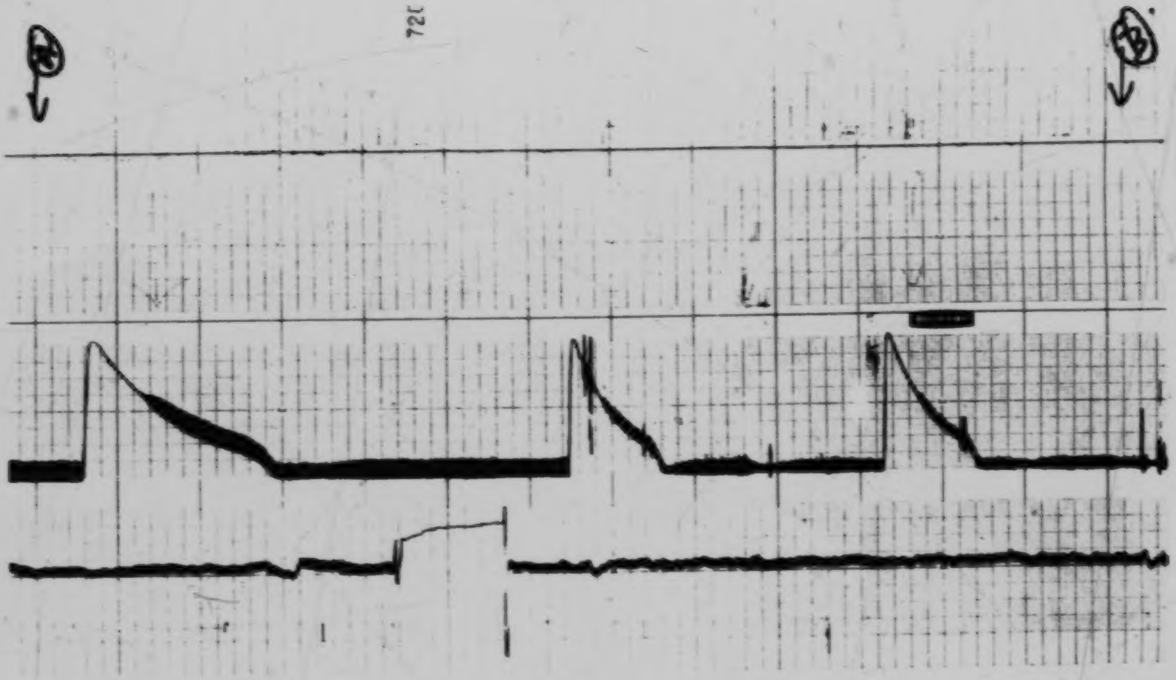

Figure 53. Cuff and cannula blood pressure measurement methods used simultaneously on the same rabbit.

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Plates

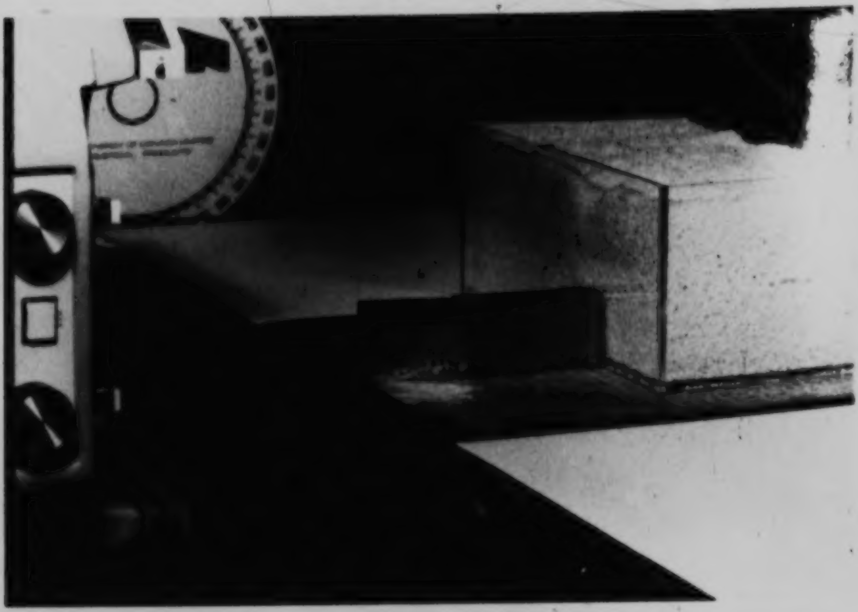


Plate 1. Lucite box filled with rice on treatment bed of radiotherapy unit. Note cerrobend blocks which are used for shielding of the heart region.

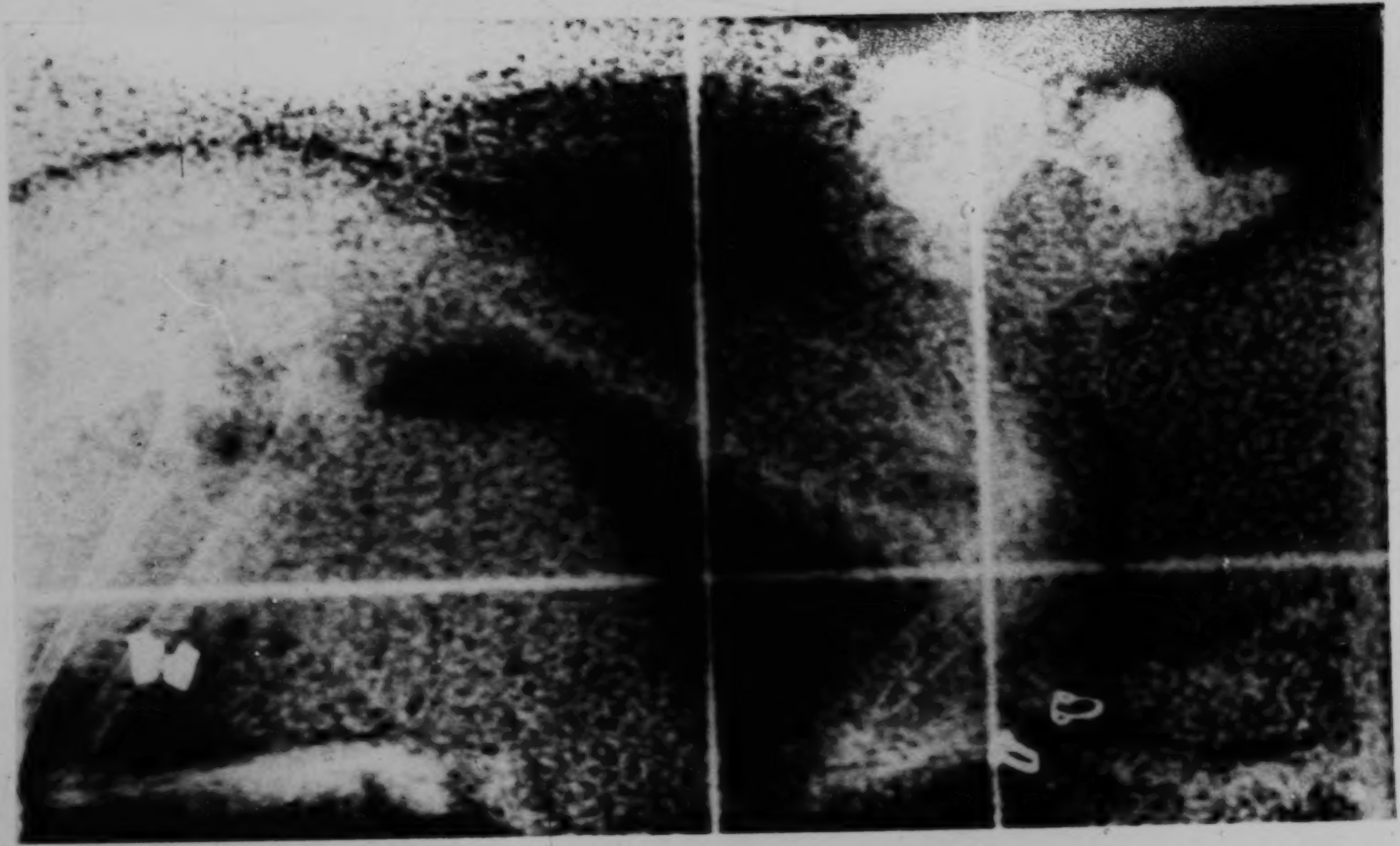


Plate 2. X-radiograph which approximates the fluoroscopic image obtained with a radiotherapy treatment simulator. Grainy appearance is due to rice packing. In practice the heart is readily localized due its pulsatile behavior.

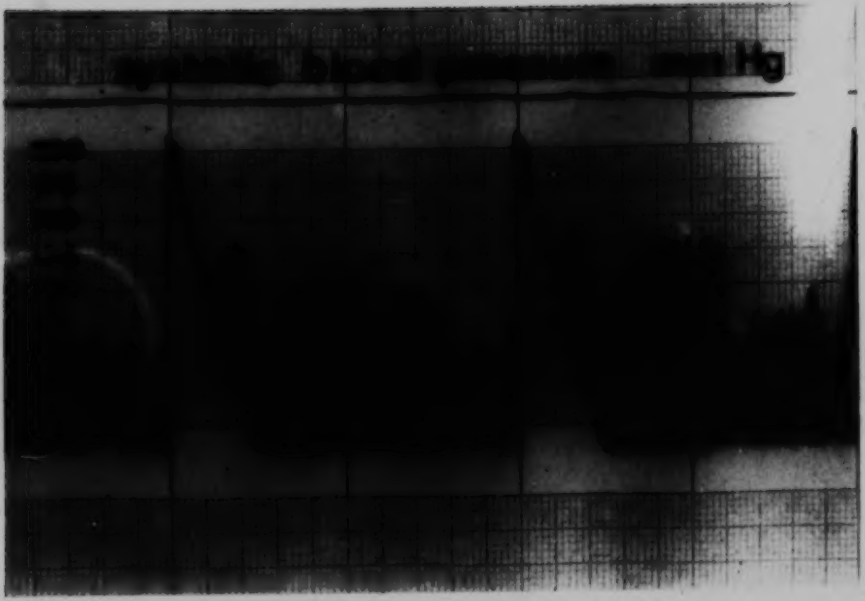
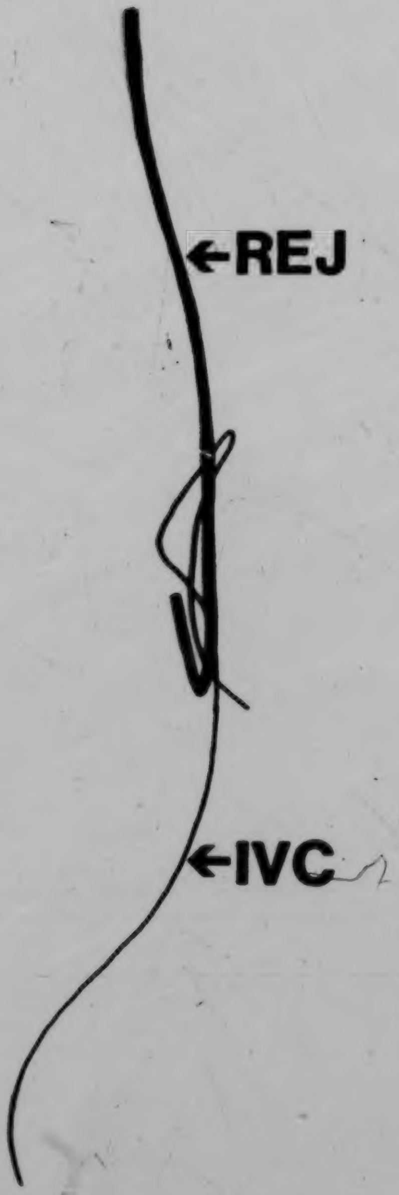


Plate 3. Photograph of a chart recording of systolic arterial pressure obtained using the tail cuff method.

Plate 4. Reproduction of an X-radiograph which was taken to produce a record that approximated the view on the screen of the mobile fluoroscopy unit during catheterization of the right ventricle. The fluoroscopy unit was switched from fluoroscopy mode to X-radiograph mode. The rabbit was on its back, and the forelimbs are clearly visible. The clear overlay shows the path of the 4F neonatal cardiac catheter through the right external jugular vein and into the right ventricle. This catheter is identified as REJ. The 3F umbilical vessel catheter can be seen entering the right ventricle via the inferior vena cava, and is identified as IVC. All right ventricular catheterized rabbits which were irradiated had the 4F catheter installed, and this was always done via the right external jugular.


←REJ
←IVC

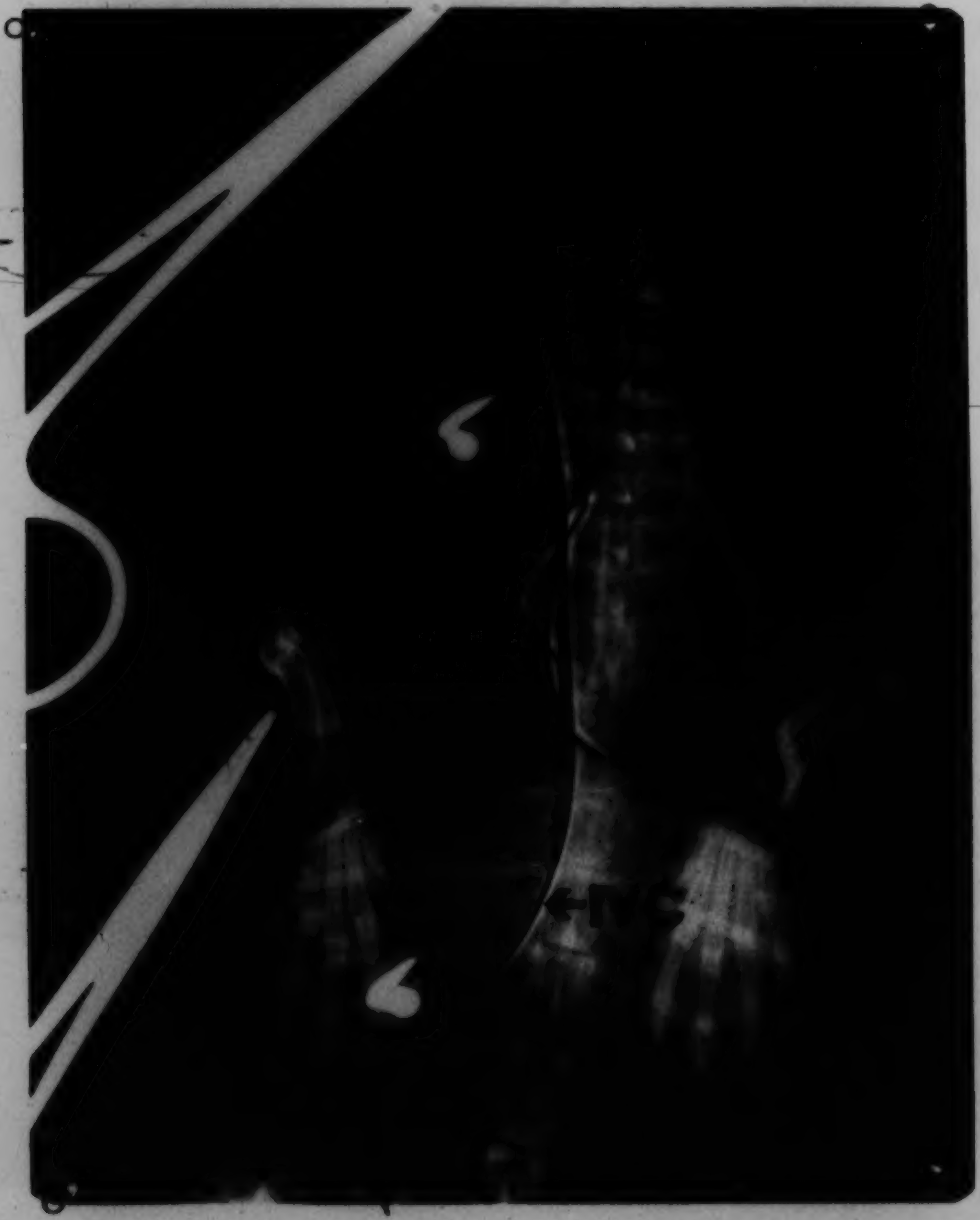


Plate 5. Photograph of the silastic nerve cuff. Platinum-Iridium wires are visible at both ends and in the center.

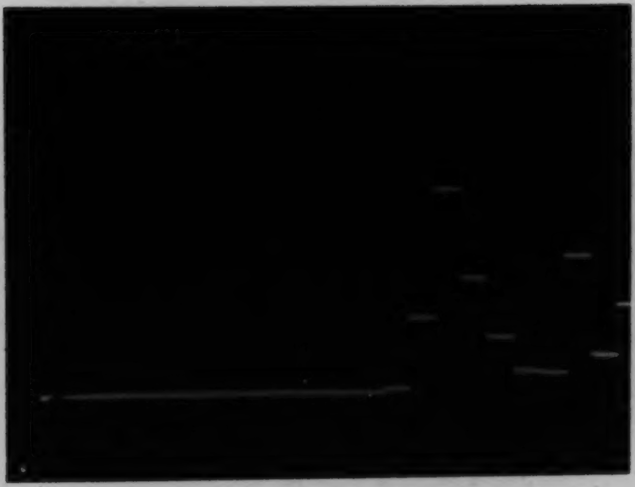


Plate 6. Oscilloscope trace of the calibration signal for neural power spectrum apparatus. Note the position of the eighth bar, on the tenth graticle line.

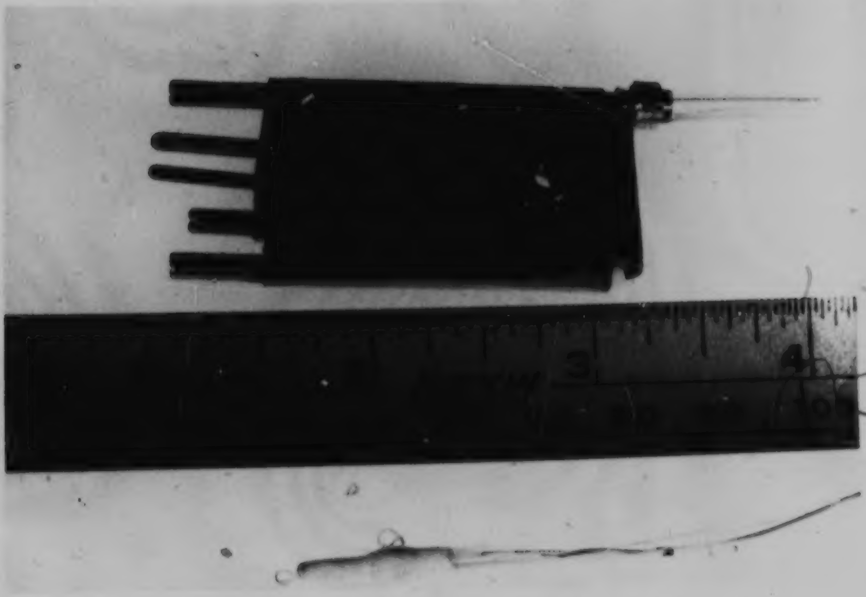


Plate 7. Vagal cooler. Connector plate is at top, and the tip cylinder, coated with white silicone rubber, is at bottom.

Plate 8. Blood pressure waveform obtained prior to 1200 cGy TBI. This was recorded using the fluid filled catheter - external transducer system in a 377 day old rabbit. The catheter was a polethylene PE-90.

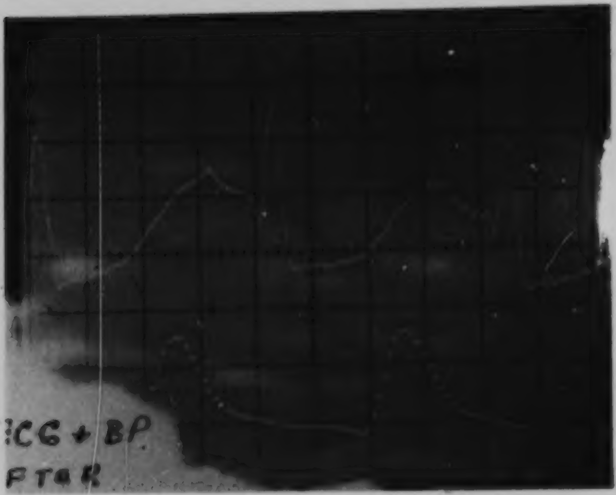


Plate 9. Blood pressure waveform from the same rabbit as in Plate 8, obtained during deliberate post-irradiation hypotension. The top waveform is the ECG, the bottom tracing is the blood pressure wave. Note the reduction in amplitude, and the absence of the dicrotic wave.

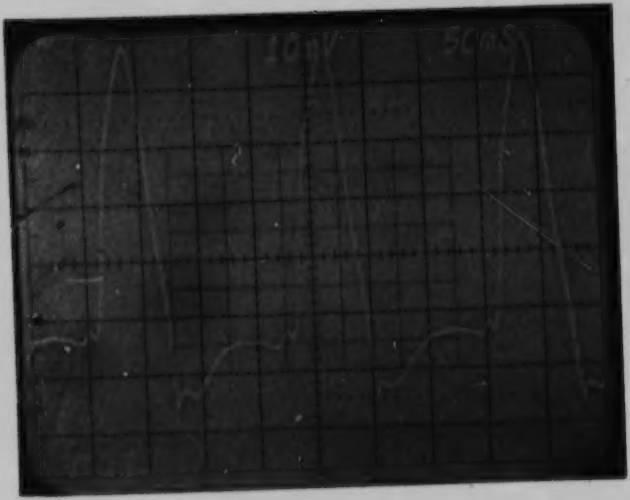


Plate 10. Blood pressure waveform obtained prior to 1020 cGy TBI. This was recorded from a 53 day old rabbit which was fitted with the Millar catheter transducer in the dorsal aorta.

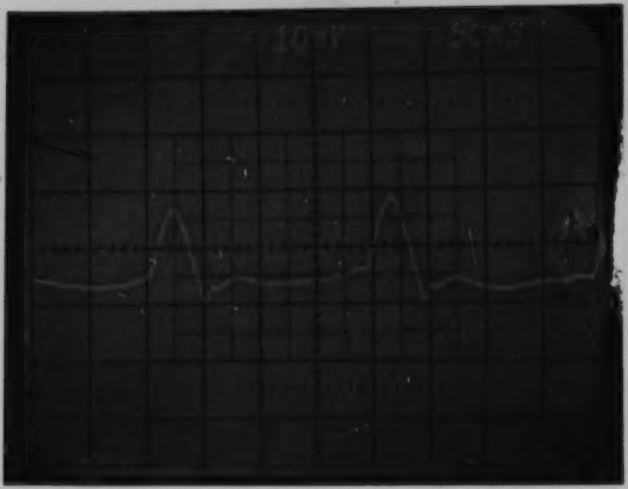
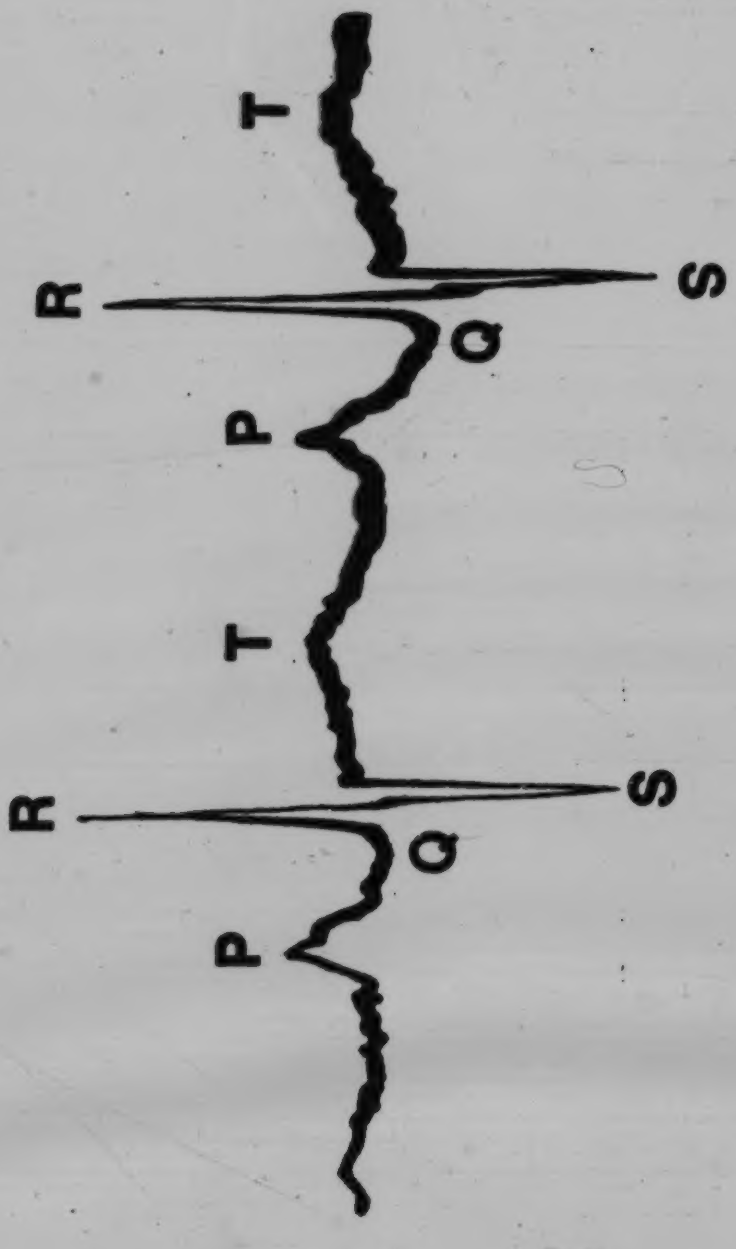


Plate 11. Blood pressure waveform from the same rabbit as in Plate 10, obtained about 12 minutes after the onset of ACC. Note the sizable reduction in both the waveform amplitude and in the dicrotic wave.

Plate 12. Electrocardiogram (ECG) obtained from a rabbit less than 100 days old. The clear overlay indicates the P wave, representing atrial depolarization, the QRS complex, representing ventricular depolarization, and the T wave, representing ventricular repolarization. Repolarization of the atria is masked by the QRS voltages.


P
Q
R
S
T
P
Q
R
S
T

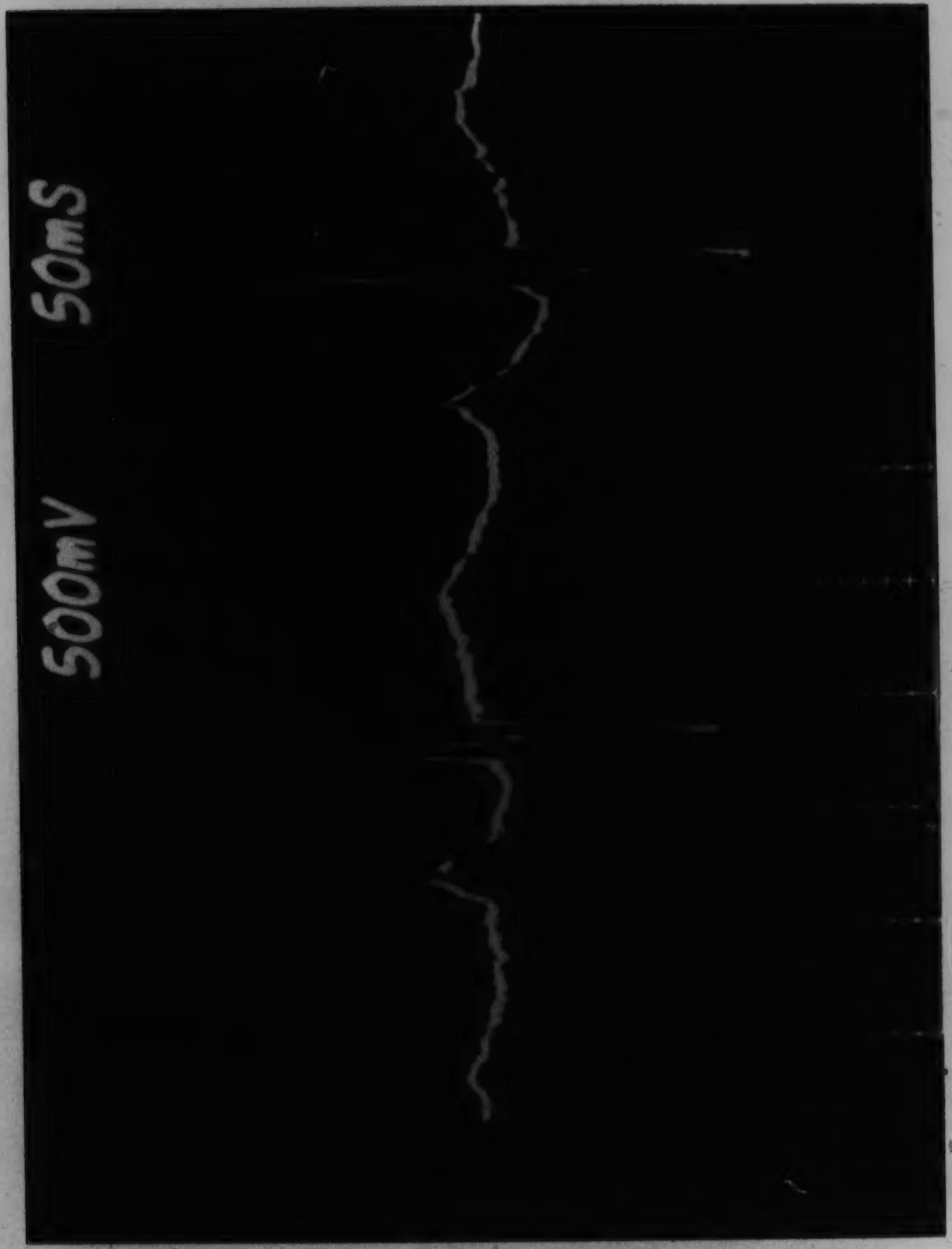
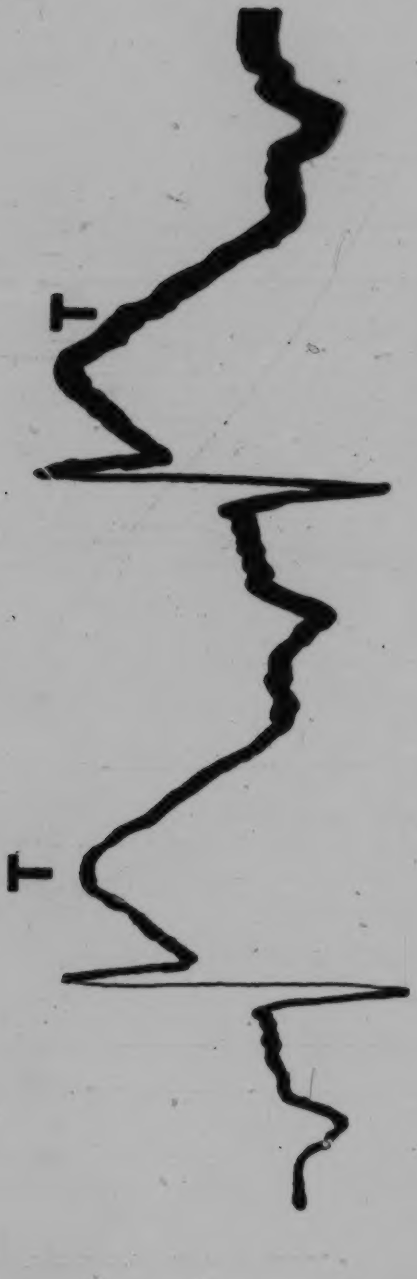
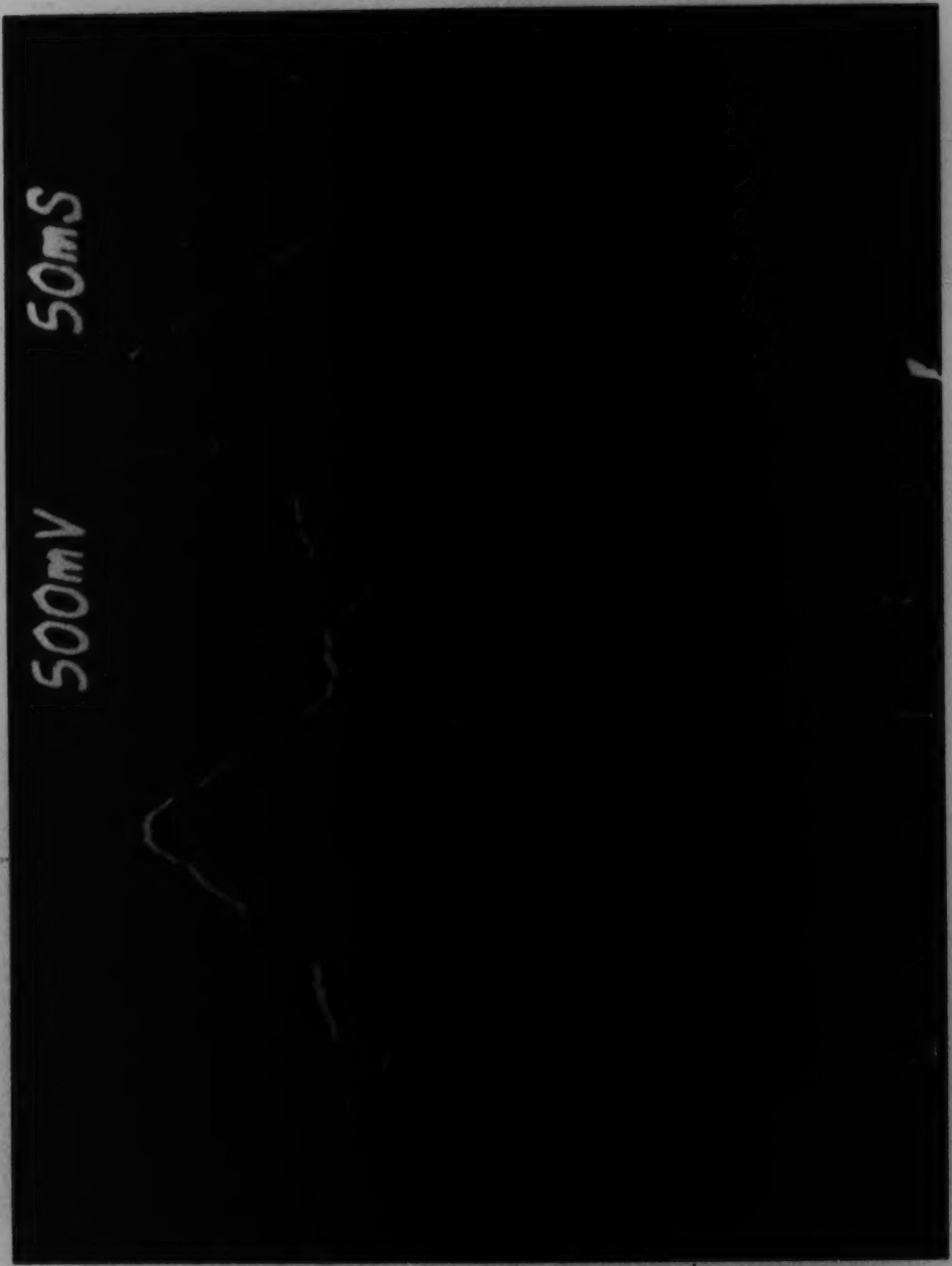

500mV
50mS

Plate 13. ECG obtained from the same rabbit as in Plate 12, just after radiation induced ACC. The most striking change is elevation of the T-wave. (Refer to clear overlay.) T-wave elevation was often seen with rabbits experiencing ACC. Also, the QRS spike is inverted from its pre-irradiation orientation, although this was not as frequent as T-wave elevation.


T
T


50mS
500mV

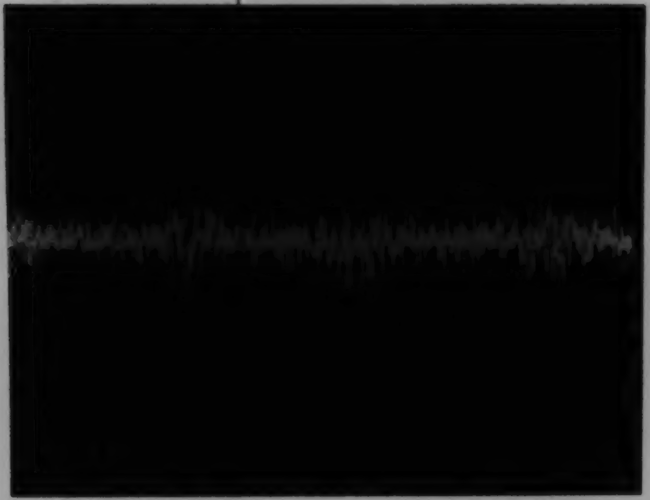


Plate 14. Time based recording of vagal signals from an anesthetized rabbit. The larger amplitude bursts coincided with breathing. The recording was obtained using silver (Ag) hook-type electrodes.

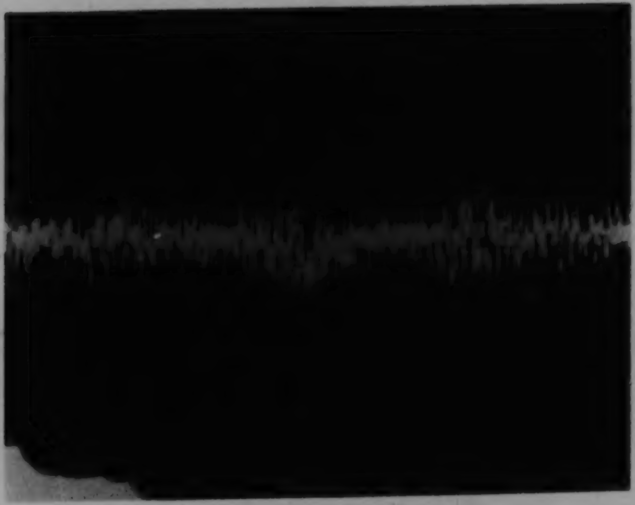


Plate 15. Time based recording of vagal signals from the same rabbit as in Plate 14. This recording was obtained with a silastic nerve cuff containing three Pt-Ir electrodes.

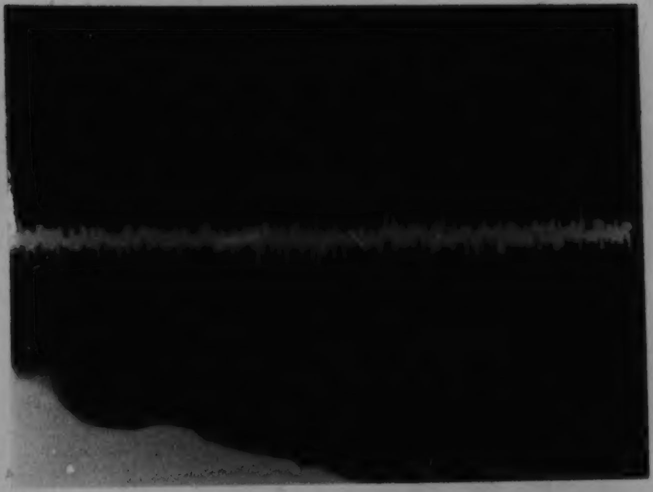


Plate 16. Time based recording of vagal signals from the same rabbit as in Plates 14 and 15. This photograph was taken after the nerve was crushed distally.

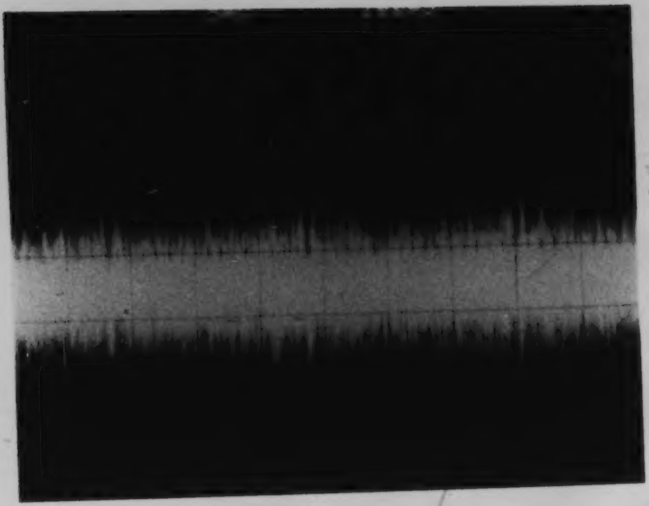


Plate 17. Time based recording of vagal nerve signals from rabbit #3PU1, obtained on the day of nerve cuff implantation. The animal was fully conscious; note the complexity of the oscilloscope tracing.

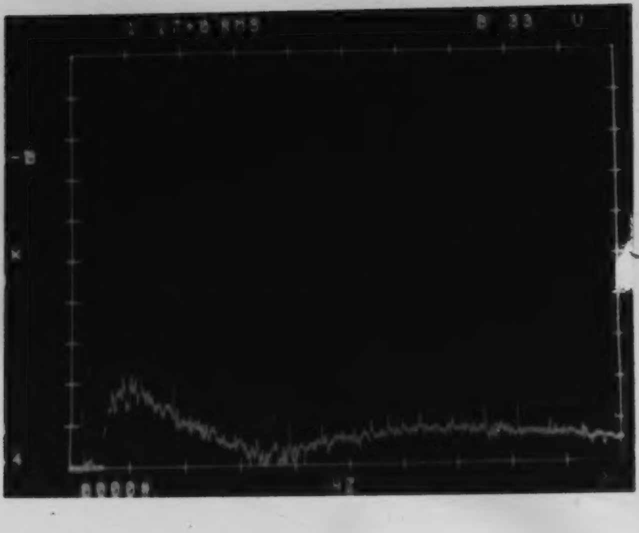


Plate 18. Power spectrum profile of vagal nerve transmission taken from the same rabbit as in Plate 17, on the same day that [illegible] 17 was photographed. Note the bipartite character of the profile.

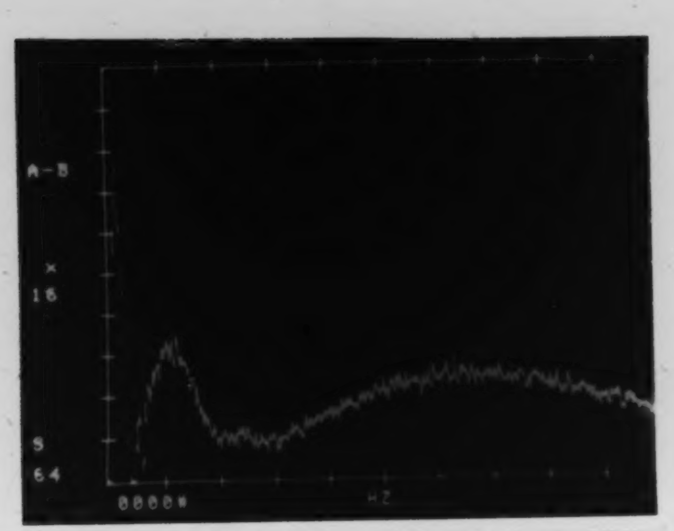


Plate 19. Power spectrum recording from rabbit #3PU1. This was taken on the day after cuff implantation.

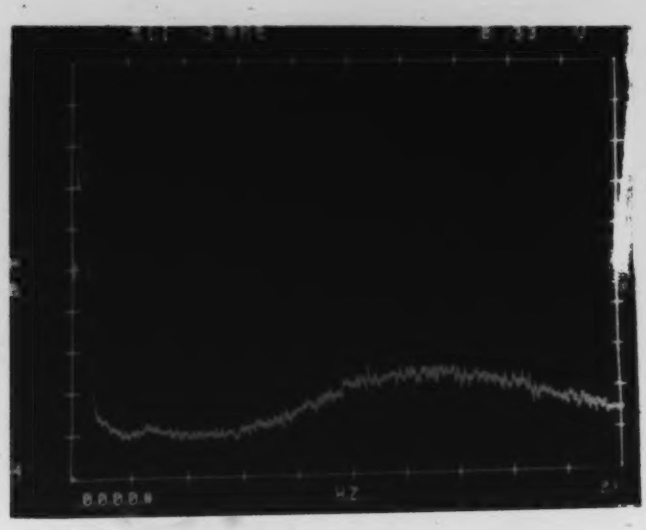


Plate 20. Power spectrum recording from rabbit #3PU1, taken just after intravenous injection with a sedating dose of sodium pentobarbital. Note the absence of the lefthand curve.

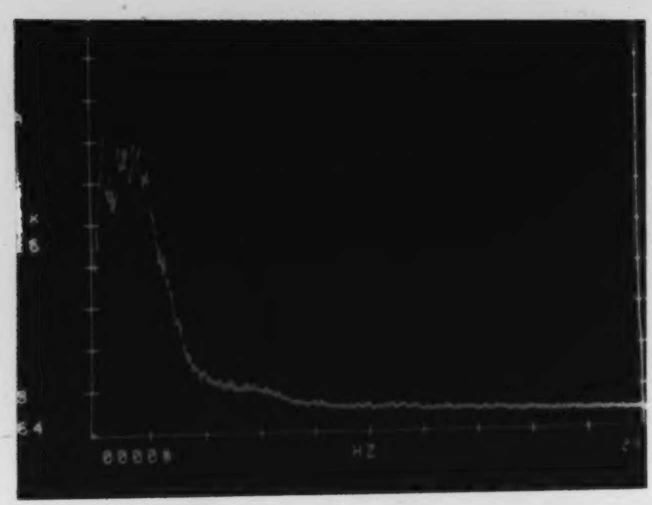


Plate 21. Power spectrum recording from rabbit #3PU1 on the fourth day after nerve cuff implantation. Note the absence of the righthand curve.

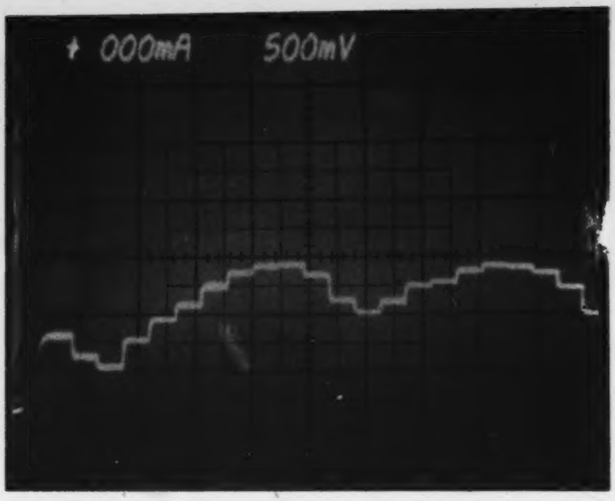


Plate 22. Power spectrum of vagal transmission from a fully concious rabbit (ID #3UO3). The central region is filled with voltage potentials which may be due to muscular activity. The putative nerve profile is visible on the right.

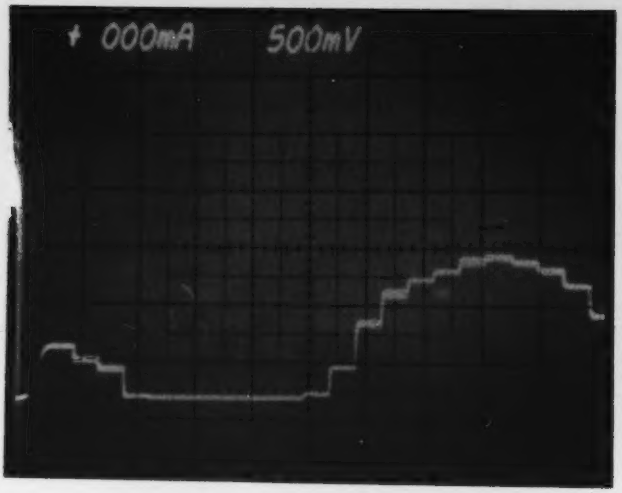


Plate 23. Power spectrum for rabbit #3UO3 one minute after a third nembutal injection. Note that the voltage potentials from the middle frequency area have disappeared.

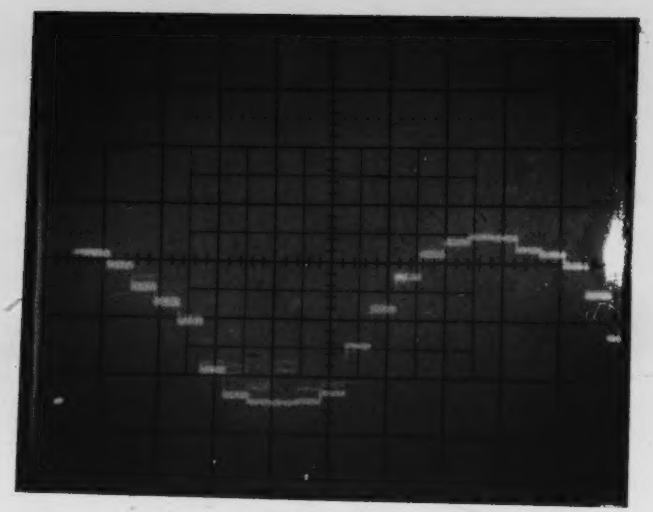


Plate 24. Power spectrum of vagal nerve transmission in a fully conscious rabbit (ID #3QU3).

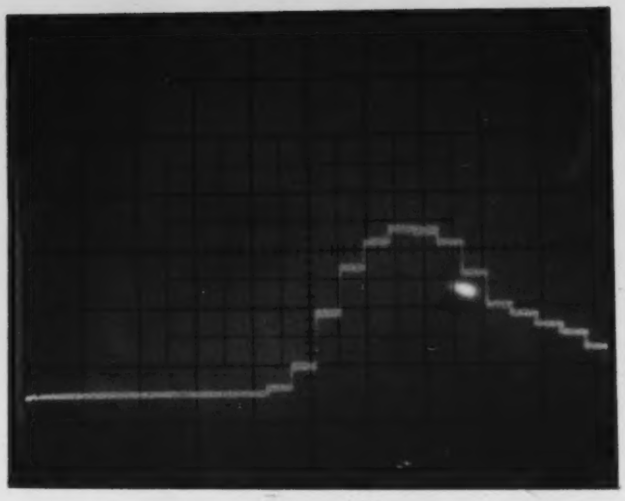


Plate 25. Power spectrum recording for rabbit #3QU3 taken just after injection with Euthanyl. The animal died instantly. The lefthand curve has vanished, while the righthand curve is clearly defined.

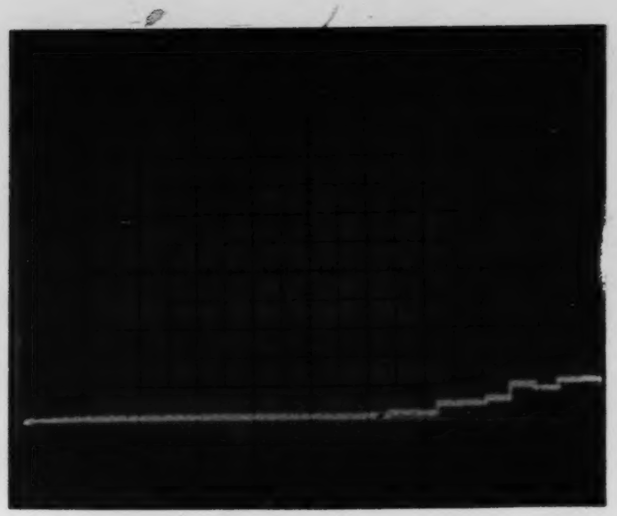


Plate 26. Power spectrum recording for rabbit #3QU3 taken at about 6.5 minutes after injection with Euthanyl. The righthand curve has nearly disappeared.

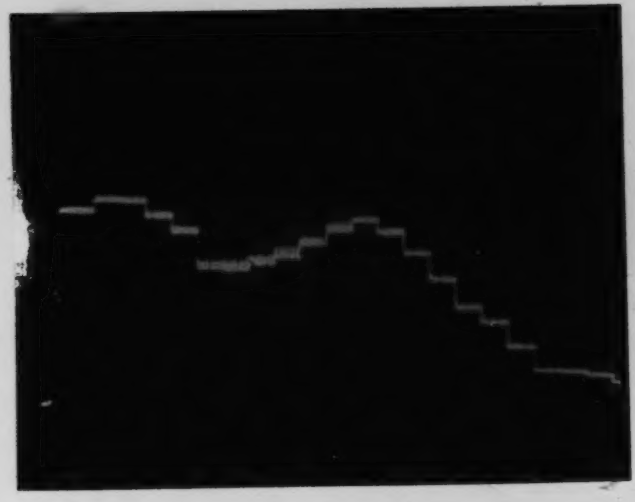


Plate 27. Power spectrum recording of left vagal transmission in a fully conscious rabbit (ID #3QN3). There seems to be little indication of a neural curve on the righthand side.

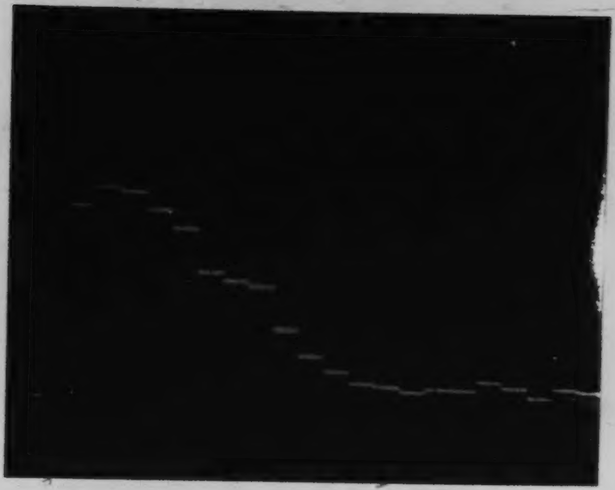


Plate 28. Power spectrum for rabbit #3QN3. The animal was fully conscious, but the voltages in the mid-region disappeared, leaving nothing in the neural frequency range.

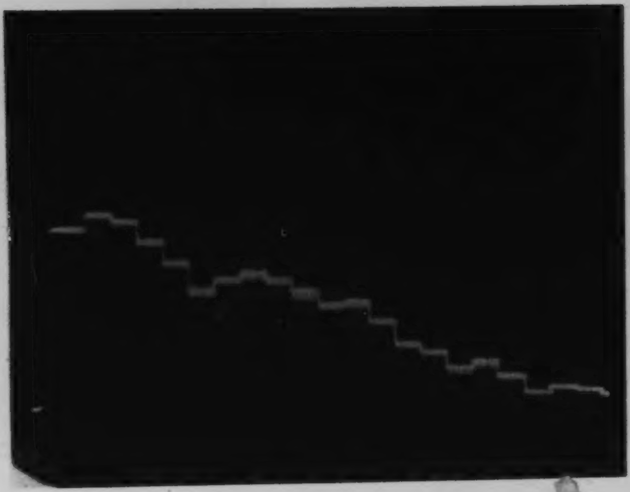


Plate 29. Power spectrum for rabbit #3QN3 taken 1/2 minute after injection with pentobarbital (Nembutal). There do not appear to be neural voltages.

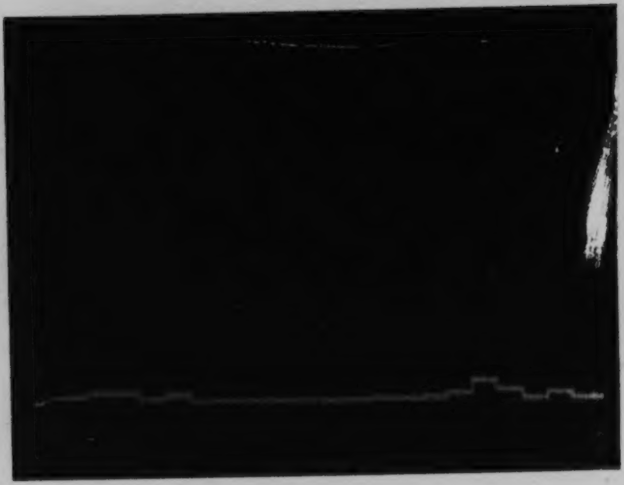


Plate 30. Power spectrum for rabbit #3QN3 taken about one minute after Euthanyl injection. Euthanyl was administered to this animal after Nembutal was injected.

Plate 31. Power spectrum of left vagal transmission in a rabbit (ID #3PS4) about 1.5 minutes after injection with Euthanyl. The rabbit was killed instantly, but there was little EMG noise from the rabbit even prior to injection.

Plate 32. Appearance of oscilloscope trace when cuff of rabbit #3PS4 was disconnected from power spectrum apparatus. The trace does not resemble that of Plate 31.

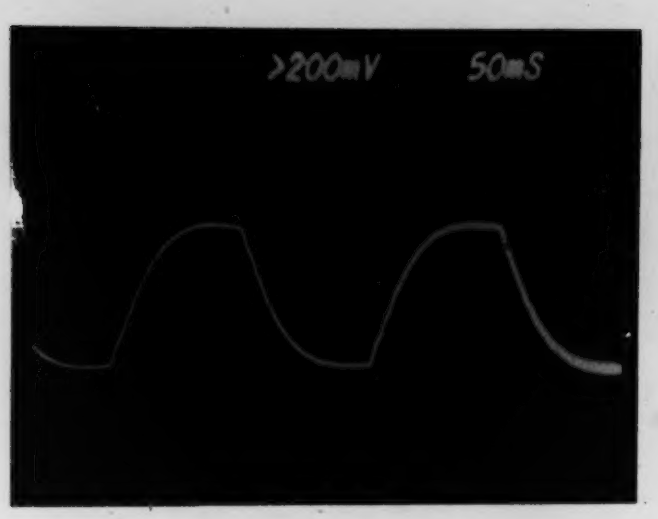


Plate 33. Response of a Gould P23 ID transducer to a 5 Hz square wave. The wave was carried by a saline filled PE-50 tube, which was connected to the wave generator and to the transducer with 23 gauge needles. The tubing was 105.4 cm long.

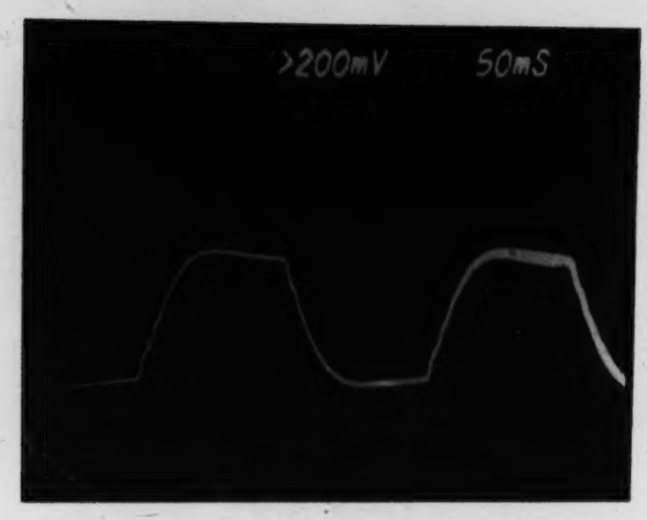


Plate 34. Response of a Gould P23 ID transducer to a 5 Hz square wave. The wave was carried by a saline filled PE-50 tube which was connected to the wave generator with a 23 gauge needle, and was fixed to the transducer with an 18 gauge needle. The tubing was 105.4 cm long.

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Appendix I

A. Accuracy of Invasive and Indirect Methods of Arterial Blood Pressure Measurement

Accuracy of Invasive Method

The external pressure transducer used with a fluid filled indwelling catheter reliably supplied accurate blood pressure measurements for prolonged intervals. Static pressure tests revealed that this system provided measurements over a wide range of pressures with little error and acceptably linearity. Regression and error calculations performed by computer on three voltages generated by the Gould P23 ID pressure transducer, in response to three supplied pressures, indicated linearity errors of less than 1%, or 2 out of 200 mm Hg. Also, comparision of static calibrations for the entire recording apparatus performed at the onset of each pressure monitoring session, with those conducted at the end of the recording intervals, revealed a generally low level of system drift, as indicated in Table X.

Arterial blood pressure in the rabbit changes rapidly, so it was necessary to ensure that the measurement apparatus was able to detect and translate changing pressure into accurately representative electrical output. Hence the dynamic tests described in Materials and Methods D were performed. The results of these tests indicated that the materials used to make a catheter, as well as its diameter and length, critically affected the performance of the measurement system. It was also found that the internal diameter of the connecting needle is an important factor.

Ultimately, the quality of the electrical output produced by a strain gauge pressure transducer, depends on the extent of transducer diaphragm displacement in response to rapid changes in applied pressure. Movement of the diaphragm causes the underlying wheatstone bridge strain gauge elements to change in length, which causes either a rise or a decline in the electrical resistance of each component. The magnitudes of the resistivity changes within the wheatstone strain gauge circuit, directly depend on the degree of diaphragm displacement, and greater displacement is scored as a greater pressure by the strain gauge amplifier and chart recorder. A sensitive transducer is one in which the diaphragm is readily displaced by very slight pressure changes, and one for which the strain gauge elements exhibit relatively large changes in resistance from very small diaphragm movements. Diaphragm displacement due to a pressure wavefront is quantitatively described by the expression $E=DP/DV$ (Gabe, 1972). E refers to the transducer diaphragm displacement, DP refers to the change in pressure, and DV is the volume change of the entire measurement apparatus, including the transducer diaphragm and conducting catheter. The extent of system volume change depends on the compliance of the member components. Compliance is defined as the ratio of change in volume to a change in pressure (Gabe, 1972), and low compliance results in a relatively large E, or diaphragm displacement, for any increase in pressure, DP. Accordingly, it was found that less compliant catheter materials, ie, stiffer tubing, improved system response. With comparatively rigid tubing less wavefront energy is dissipated in catheter wall distention, and hence more energy reaches the transducer so that greater diaphragm displacement occurs. A catheter with comparatively low compliance possesses high resonant frequency, which raises the resonant frequency of the entire measurement system (Shapiro et al, 1970). Thus the system will only resonate to the higher frequency elements of the primary pressure waveform being measured. The higher the frequency of the individual component involved, the less energy it contributes to the overall measured waveform, and the extent of system resonance, or ringing, will be relatively less.

It was found that polyethylene tubing (Clay Adams, New York, NY) provided an appropriate degree of stiffness, yet was sufficiently flexible to permit surgical implantation, and subsequent movement by the rabbit without traumatizing tissue.

The use of immature rabbits from a physically small breed imposed restrictions on the size of catheter tubing which could be implanted. Small diameter catheters present a relatively high frictional resistance to pressure waveform propagation, and consequently a relatively high degree of damping of the pressure wavefront occurs (Shapiro and Krovetz, 1970). Overdamping or underdamping both increase the effective response time of the system, and both distort the pressure waveform. (Note, "response time" here refers to the time required for the waveform to attain a stable final value, while "rise time" refers to the time needed to go from the 10% point of the rising waveform, to the 90% level.) A beneficial effect of the relatively high level of signal attenuation inherent with bore tubing, is that ringing of the system is reduced, resulting in less distortion from resonance (Manktelow and Baird, 1969). Figures 49 and 50 depict the rise times to a square wave of pressure for a measurement system incorporating, respectively, PE-10 and PE-90 tubing. The rise time of 178.4 ms with PE-10 tubing (ID 0.28 mm) is considerably inferior to the 9.2 ms rise time with PE-190 tubing (ID 1.19 mm). However, these figures also show that PE-190 tubing is more prone to ringing even though its natural reasonant frequency is higher, ie, the system is underdamped. But, the narrow bore of the PE-10 cannula, which diminishes (damps) ringing, in addition to reducing response time, aslo causes attenuation of the high frequency components of a pressure waveform, resulting in erroneously high diastolic and low systolic pressures to be recorded, as illustrated by Figures 51 and 52. The amplitude of the continuous sine wave recording, which mimics an aortic blood pressure record, is considerably greater with

PE-190 tubing than with PE-10.

Ideally, the damping factor should diminish system ringing without markedly slowing response time (Gabe, 1972), and should not excessively attenuate the pressure wavefront. In the present study it was found that PE-50 (ID 0.58 mm) and PE-90 polyethylene tubing (Clay Adams, New York, NY) facilitated acceptable system response. The final and the easiest measure of tubing performance was empirical, and good response is represented by the relatively wide amplitude chart tracings of blood pressure taken for many rabbits. It was found that the small bore steel connecting needles used with PE-50 cannulae damped the pressure wavefront more than larger diameter needles. This is shown by Plates 33 and 34. System response time with a PE-50 line connected to a 23 gauge needle (nominal ID 0.0125") at the transducer end is much poorer than when the same tubing is connected to the transducer via an 18 gauge needle (nominal ID 0.033"). Therefore, about midway through the project it became practice to connect the aortic catheter to the external transducer via an 18 gauge needle: a stepwise series of short polyethylene tubes facilitated connection of the PE-50 or PE-90 catheter to the larger needle.

Shortening the length of catheter tubing between the rabbit and pressure transducer improved system response (Shapiro and Krovetz, 1970). The basis for this is that wavefront energy loss inevitably occurs with all catheters: the greater the tubing length, the the greater the total energy loss. Thus for all experiments catheter length was kept to a minimum. It was also important to remove air bubbles from the catheter and the transducer dome prior to system calibration and blood pressure recording. Air bubbles increase system compliance and transmit pressure changes very poorly (Mankeltow and Baird, 1969). They also lower the resonant frequency of the measurement system so that distortion due to ringing is greater--- even very small bubbles can severely degrade pressure recording (Shapiro and Krovetz 1970). Hence the transducer dome volume, system lines, and catheter were routinely inspected for the presence of trapped air, and the entire system was often pressurized and the forward transducer stopcock opened to allow reservoir water to flush through, thereby expelling bubbles. Early in the project, water was boiled before being added to the external transducer system, and mineral oil was poured on top of the reservoir jar water to block entry by atmospheric gases. However these precautions were eventually omitted because air bubbles rarely formed, even without the layer of mineral oil, and because system flushing facilitated good quality blood pressure measurements regardless of whether or not the reservoir water was boiled.

The level of blood pressure waveform detail provided by the external transducer system was compared against waveforms supplied by an indwelling catheter transducer. The shape of the aortic waveform can reflect the extent of vasodilatation in peripheral vascular beds--- widespread vasodilatation can produce significant hypotension--- so accurate recording was deemed important for this study (Langille, 1975). The catheter transducer (Mikro Tip, Millar Instruments) was implanted in the dorsal aorta and thus avoided the drawbacks associated with a fluid filled conducting cannula, viz., the loss of waveform energy through damping, compliance, air bubbles and blood clots. The second important feature of the Millar transducer is that its resonant frequency range lies at relatively high frequencies, viz., 25 kHz to 40 kHz. This range is far removed from the most energetic harmonics of the blood pressure waveform, so that the degree of catheter ringing, and the extent of distortion due to ringing, ought to be comparatively low. The frequency response range of the Millar catheter system (transducer and TCB-100 amplifier) is 0 Hz to 8 kHz. A third significant advantage of the catheter transducer is that it possesses a very small diaphragm. The low mass and low displacement volume of this diaphragm allow weak, rapid pressure changes to be more easily detected. Since the catheter transducer is highly responsive and eliminates the restrictions imposed by a fluid filled cannula, it constitutes a good standard by which to evaluate the performance of the external transducer system. However, the latter was used for the overwhelming majority of rabbits because the catheter transducer is too large to be implanted in most weanling Dutch belted rabbits, is of limited durability and is costly.

Plates 8 through 11 show that the general waveform shape during normal pressure and during hypotension was similar using the external transducer and the catheter transducer. The dicrotic wave was prominent for normotensive rabbits using either transducer system--- this degree of waveform detail meant that the external transducer system was capable of discerning waveform changes associated with peripheral vasodilatation. According to Manktelow and Baird (1969), the presence of a dicrotic notch indicates that the blood pressure measurement system is supplying adequate performance. The dicrotic notch is visible in Plates 8 and 10. Furthermore, the blood pressure tracings obtained following irradiation were similar in terms of the general shape of precipitous pressure declines using the two methods, indicating that in practice, the external system measured rapid change and did function for low arterial pressures (Plates 9 and 11). Also, since the catheter transducer obviated the need for flushing, and since hypotension with characteristic pressure waveform changes following irradiation were detected by this device, clearly the post-exposure alterations recorded in the blood pressure waveform using the external transducer were not artifacts elicited by infusion of heparinized saline. Difficulties with the external transducer system did arise and on occasion completely interfered with blood pressure recording. Blood clots sometimes formed at the tip of and within the catheter, periodically resulting in blockage. If the cannula could not be reopened by flushing, blood pressure measurement could not proceed. In a few rabbits it seemed as though the catheter tip was lodged against the vessel wall, causing partial or complete interruption of pressure transmission. Late in the study, the cannula was coated prior to implantation with silicone lubricant to prevent sticking and jamming into the wall of the femoral artery or dorsal aorta.

Accuracy of the Tail Cuff Method of Blood Pressure Measurement

The tail cuff method and the external transducer approach were employed simultaneously in two animals, and demonstrated that the cuff technique provided a sufficiently accurate measure of systolic blood pressure, both before and after irradiation. Also, system drift was low, certainly within acceptable limits, as shown in Table X. Although the cuff method tended to underestimate pre-irradiation and post-irradiation systolic pressures, general pressure trends were recorded (Table XI, Figures 15 and 53).

Two possible causes for the discrepancy in pressure readings between the external transducer and tail cuff methods may have been: (1) the cuff technique systematically provided low measurements to due limitations of this technique, such as inability to detect very faint tail changes associated with the resumption of blood flow after occlusion, or difficulty in maintaining constant, correct pressure between the tail and pulse transducer; and (2) general anesthesia, implantation, and presence of an indwelling catheter in the dorsal aorta might have elicited higher than normal aortic pressures in operated rabbits.

After testing the cuff on the forelegs, hindlegs and ears of adult and immature rabbits, and following the advice of several personal communications, it was found that the consistently strongest signals could be detected from the shaved tail. The pulse detector lead and air line were made of flexible materials so that the tail, pulse transducer, and cuff moved together unhindered. Otherwise, if the leads were too stiff the the tail tugged on them. This caused the pressure attachments to move relative to the tail, thereby producing noise and interfering with the pulse signal. The cuff was made short to allow adequate space on the tail for it and the pulse transducer. Several tests were performed before the final combination of air pump and amplifier instrumentation was determined. The Narco cuff pump was selected for its capacity to almost instantaneously inflate the cuff to 200 mm Hg pressure; it was learned empirically that slow cuff pressurization resulted in poor measurements. The Beckman cuff amplifier is a compact device which translated the air pressure and tail pulse information into an easily readable chart tracing. Dr. J. Russell of the Department of Surgery at the University of Alberta kindly recommended the Beckman amplifier for tail cuff monitoring, and he also helped test this device on the rabbit.

Appendix II

A. Results of Exploratory Chemical Tests

S-Beta, 2-Aminoethylisothiuronium Bromide Hydrobromide - AET

The sulphydrl radioprotective compound AET was tested in rabbits to ascertain whether it could provide at least some protection against radiation hypotension. Prior to such experiments however, the maximum safe levels of AET had to be determined using toxicity tests with Coturnix quail and rabbits. The 24 hour mid-lethal dose for AET without NaOH, at a pH of 4.5 to 5.5, was about 215 mg/kg, while the 24 hour mid-lethal dose of AET neutralized with NaOH (pH 7 to 8), was about 340 mg/kg. This was unexpected since it has been reported that that AET at low pH is more toxic than solutions at neutral pH (Khym et al, 1957). Apparently this is because a significant proportion of AET at low pH transforms to 2-aminothiazoline (2-AT) which is more toxic than mercaptoethylguanidine (MEG). MEG is the major transformation product at neutral pH. It was found that rabbits could tolerate doses of 200 mg/kg and 300 mg/kg of AET injected intraperitoneally. When 400 mg/kg was given the animals died within 24 hours. Rabbits did not die within the first week of injection with 200 and 300 mg/kg of AET, but one rabbit injected with 300 mg/kg died at 9 days. The body cavity was examined and the most notable finding was that the kidneys and small intestine were swollen. A study in which mice were injected with isotopically labelled AET indicated that the drug concentrates in these organs within hours of injection (Maisin et al, 1965). Microscope examination revealed that the kidney tubules seemed to be surrounded by unusually large spaces. Apparently these spaces were filled with a fluid which contained a moiety which was fixed and stained. The villi of the small intestine were not well defined, and cells appeared to be sloughing off. Nevertheless, the absence of early, obvious toxicity allowed 200 mg/Kg of AET to be given prior to irradiation, for initial tests of activity against radiation hypotension. When 112 mg/kg of unneutralized AET was injected intravenously in six nonoperated rabbits 20 minutes prior to TBI, it was found that the survival of these animals was not greater than that of non-treated, TBI rabbits. One cannulated rabbit was injected with 56.9 mg/kg AET intravenously, and died within minutes. Another cannulated rabbit was injected intravenously with 108.7 mg/kg of AET 20 minutes prior to total body exposure in the Gammacell 220 to 1111.2 cGy at 1481.6 cGy/minute. This animal lived about 15 hours after irradiation. Four rabbits were injected intraperitoneally with 200 mg/kg of neutralized AET 20 minutes prior to 1200 cGy TBI. In one of these rabbits it was thought that a significant fraction of the drug may not have entered the peritoneal cavity; this was uncertain. None of the rabbits exhibited the acute fall, and the blood pressure declined by 29.1% (SE=16.02). However, too few rabbits were tested to draw any conclusions. Ms. D. H. Geibelhaus found that IM injection with 15 and 25 mg/kg before TBI did not prevent acute hypotension, and the extent of hypotension was unaffected, at 58 to 75%. The rabbit injected with 70 mg/kg both before and after TBI showed a deliberate pressure decline with a total drop of 30.5%. Nine rabbits were injected with 30 mg/kg before TBI. Three of 8 rabbits exhibited acute hypotension (the recording for one rabbit was unclear), and the average decline for the entire group was 44% (SE=8.49, n=8). One of 8 rabbits showed the acute drop but within a few minutes the pressure fully recovered. The one rabbit injected with 30 mg/kg experienced a deliberate decline of 19.4%.

Nine cuff monitored rabbits were injected IP with either 200 or 300 mg/kg of AET either 20 or 30 minutes prior to TBI. With 5 of the rabbits the cuff derived blood pressure recording was very poor, so that the results could not be interpreted for these animals. Three of these animals exhibited a marked loss of abdominal muscle tone during the recording session. Good pressure records were produced for 4 of the 9 rabbits, and in 2 of these cases acute hypotension was noted. The results from this work cannot be definitely interpreted, but it appears that AET did not abolish acute hypotension. The difficulty in obtaining good cuff pressure records with AET injected animals may stem from the fact that this drug has considerable cardiovascular effects. In cats AET and 2-AT have been found to elicit first a fall and then a rise in arterial pressure (DiStefano et al, 1956; DiStefano et al, 1959). Also, following AET injection the rabbits tended to tense the voluntary musculature and seemed agitated.

A major difficulty encountered with AET was that the drug was very hygroscopic, and although it was stored in a desiccator, it seemed to absorb moisture when even briefly exposed to the atmosphere. Also, the suppliers did not guarantee that the drug would be anhydrous. When exposed to moisture, the drug eventually degrades to 2-AT which is reported to be less radioprotective and more toxic than AET (Doherty, 1960). When selecting AET for experimentation, lumps were discarded, and the most powdery from of the compound was prepared for injection.

Acetaldehyde

The 24 hour mid-lethal dose of acetaldehyde injected intraperitoneally in Japanese quail was 8 mM/kg. In rabbits acetaldehyde was considerably more toxic, and it was found that the animals could tolerate 1.7 mM/kg, which is about 154 mg/kg.

Acetaldehyde and AET Injected Together

The immediate toxicity of AET is produced by its extracellular, cardiovascular effects. It was hoped that by administering AET together with acetaldehyde that the two compounds would form a weak physical pairing, and that AET would not be free until it entered the cell. In this way it was reasoned that the immediate toxicity of AET would be bypassed, allowing relatively high doses of the drug to be administered, thereby increasing the level of radioprotection. It was reckoned that the acetaldehyde would by safely metabolized by the cell, as is the case when it is produced in humans following consumption of alchohol (Victor and Adams, 1974). Howevever, when Japanese quail received 190 mg/kg of AET together with equimolar acetaldehyde (solution neutralized with NaOH) the toxicity was greater than with 190 mg/kg of neutral pH AET alone. Also, when 300 mg/kg of AET was injected with double the concentration of acetaldehyde (2.2 mM/Kg) at neutral pH, the toxicity was the same as when NaOH neutralized AET was given alone. Acetaldehyde does not reduce the toxicity of AET

Histamine

Histamine was injected intravenously in 2 rabbits to determine if labored breathing and prostration similar to that seen during acute radiation shock would be induced. Only a small fraction of the planned doses of 7.8 and 1.8 mg/Kg were injected before the aninimals died. This result only indicates that the rabbit is quite sensitive to histamine, and that very low doses must be tested to determine if histamine injection can mimic radiation shock.

Daunorubicin

Daunorubicin belongs to the anthracycline group of antibiotics which are effective anti-cancer agents (Goodman Gilman et al, 1980). However, a limitation of these drugs is that severe cardiomyopathy can develop following treatment. Treatment with daunorubicin has resulted in an abnormal ECG, fibrosis in the atria of the heart, and damage to cardiac neurones (Smith, 1969). In humans, transient ECG ST-T wave changes have been noted several days after administration of adriamycin (doxorubicin), a compound which is related to daunorubicin, and which is similar in terms of its toxicity. Cardiomyopathy often develops some time after the ECG changes, leading to death in a significant proportion of patients (Blum and Carter, 1974; Chabner et al, 1975). The present study revealed that irradiatio of the heart region alone leads to hypotension and ECG T-wave elevation, and it was postulated that daunorubicin, because of its cardio-toxicity, might cause the same cardiovascular consequences in the rabbit system. Hence, a rabbit model could

could serve as an early test for this toxicity. Two rabbits showed blood pressure declines of 8.7 and 13.5% after daunorubicin doses of 37.3 mg/kg and 10 mg/kg, respectively. No ECG T-wave elevation was observed. The level of hypotension recorded following intravenous daunorubicin was about the level that some sham irradiated rabbits displayed, suggesting that the drug had no immediated blood pressure effect.

Enzyme Inhibitors

Some workers have postulated that tissue damage caused by radiation results in the liberation of lytic enzymes which might damage tissues and erode blood vessel walls (Rigdon and Curl, 1943; Mount and Bruce, 1964; Jolles and Harrison, 1966; Stearner and Azuma, 1968; Eassa and Casarett, 1973). The latter effect could result in extensive extravasation and a drop in blood pressure. Therefore, several enzyme inhibitors were assayed for protective activity against radiation hypotension.

Soyabean trypsin inhibitor (SBTI) did not protect irradiated chicks against early mortality; 2 of 3 birds died within about 5 hours of irradiation. One of the SBTI treated, irradiated chicks had to be killed with euthanyl due to injury. One saline injected, chick died within roughly 5 hours of TBI, and 2 other saline injected chicks were killed with euthanyl at about 5 hours after TBI. SBTI has been reported to abolish the concentration of dye in irradiated areas of skin on intact rabbits (Jolles and Harrison, 1966). Prevention of lethal radiation shock in chicks by injection with SBTI has been reported by Stearner and Azuma (1968). However, in those experiments the drug was given intravenously, while in the present study the SBTI solution was injected intraperitoneally due to difficulty with the venous route. One rabbit was injected with SBTI through a cannula which had been earlier implanted in the dorsal aorta for blood pressure measurement. This animal experienced acute hypotension, with a decline in mean aortic pressure of 62.9%. At the time of the acute drop, the ECG T-wave and ear temperature elevated rapidly.

The polypeptide protease inhibitor, Trayslol, was 298 days past the package expiry date when given to a pair of rabbits. However, the supplier indicated that the efficacy of the preparation would not be seriously diminished (Shah, 1987). Intravenous injection with 60,000 KIU of the drug in a non-operated rabbit produced no obvious constitutional or behavioral effects over several hours. An operated rabbit injected intravenously with 20,000 KIU prior to TBI experienced acute hypotension, ECG T-wave elevation, and a sudden rise in the ear temperature. The decline in mean aortic pressure was 62.8%.

Epsilon-amino-N-caproic acid (EACA) has been demonstrated to abolish the blush of vital dyes in irradiated rabbit skin following various doses of low energy X-rays (Jolles and Harrison, 1966; Eassa and Casarett, 1973). Thus, EACA, 800 mg/Kg, was injected intravenously 30 minutes prior to whole body irradiation, in a single rabbit which was cannulated in the dorsal aorta. This animal experienced acute radiation hypotension, but neither exhibited ECG T-wave elevation nor a jump in the ear temperature. The decline in mean aortic pressure was 47.8%.

Mechloroethamine

The nitrogen mustards have been termed "radiomimetic" chemicals; that is, injection with these compounds can cause some of the toxic effects on rapidly proliferating tissues, such as the hematopoetic system, as does radiation exposure (Elson, 1963). These drugs, and especially mechloroethamine have powerful CNS effects, and elicit severe nausea and vomiting when given to cancer patients (Goodman Gilman et al, 1980). In this study, it was reasoned that perhaps mechloroethamine injection in rabbits could elicit hypotension and ECG alterations; thus the rabbit might serve as a useful test system for nitrogen mustard toxicity. It has been reported that mechloroethamine injected in cats at 0.002 mM/kg caused copious salivation, paralysis of the voluntary muscles, and a fall in arterial blood pressure (Hunt and Philips, 1949). The response of the vagus to electrical stimulation was abolished after mechloroethamine was injected. When the drug was given at 0.01 mM/kg, the arterial pressure rose. The authors concluded that these results indicated that the drug has powerful CNS effects.

O'Connell and Berenbaum (1974) injected dogs with cyclophosphamide, a drug which belongs to the nitrogen mustard class, but which has less severe CNS effects than mechloroethamine. However, patients treated with cyclophosphamide do experience nausea and vomiting. (Goodman Gilman 1980) The dogs all died within 6.5 hours of injection with 500 mg/kg of cyclophosphamide. During the 1-2 hours before death the animals exhibited arterial blood pressure falls of 25-80 mm Hg, and prior to the onset of hypotension, had labored breathing. The central venous pressure increased after injection. Upon autopsy the heart was swollen and flabby, with evidence of myocardial damage, and there was pulmonary edema. The liquid was frothy, white, and did not contain red blood cells. The ECG showed lengthening of the Q-T interval, and changes in the T-wave. The authors concluded that death was due to myocardial damage, and also possibly from pulmonary edema arising from damage to the lung capillaries.

Mechloroethamine was injected in non-operated rabbits to determine whether similar reactions to those occurring within 90 minutes of whole body irradiation developed. Twenty mg/kg was the minimum dose at which an early effect was noticeable; sluggishness was observed. The rabbit given

27 mg/kg, and one of the two animals receiving 50 mg/kg, experienced immediate prostration; one of the 50 mg/kg rabbits died overnight. However, at doses below 20 mg/kg the rabbits showed no response at all within the first few hours; they only seemed be ill affected when the dose was much greater than that used in cancer patients. The maximum clinical dose of this drug is usually 0.4 mg/kg[32] for a single course of treatment, while the rabbits seemed to be affected only when 20 mg/kg, or higher, was given. Nevertheless, it may still be worthwhile to test for any cardiovascular effects of nitrogen mustard injection, using rabbits catheterized in the dorsal aorta. In the present study, other experiments, such as heart irradiation, took precedence when the preliminary mechloroethamine tests were not very promising.

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[32] From Goodman Gilman et al, 1980.

Appendix III

A. Irradiation of the Liver with the Gut, and of the Upper Half Body

The methods and results for irradiation of the liver with gut, and irradiation of the upper half body are placed outside the body of the thesis, and are not shown in Figure 20, because only one rabbit was used for each experiment. Such a small number was tested due to attention rapidly focussing on other experiments.

Irradiation of the Liver and Gut

A 50 day old rabbit was starved overnight, anesthetized with halothane, ECG electrodes attached, and a catheter containing heparinized 0.9% saline implanted in the dorsal aorta; all according to the methods explained in Materials and Methods D. After a recovery period lasting several hours, the rabbit was taken to the RRC and the aortic blood pressure, ECG, and ear temperature were recorded. The animal was then taken to the CCI and its internal anatomy was examined fluoroscopically. The area to be exposed was marked on both longitudinal sides of the lucite box. A 4 by 4 cm square, horizontal beam produced by the 6 MeV linear accelerator, provided 1200 cGy at a dose rate of 200 cGy/min to the liver and anterior portion of the gut region.

The rabbit was then transported back to the RRC, and physiological recording was resumed for 104 minutes. The lowest post-irradiation mean aortic pressure was 17.8% lower than the lowest pre-irradiation mean pressure. No ECG T-wave elevation was seen, and the ear temperature was not measured. At the end of the recording session the rabbit was killed with pentobarbital.

Upper Half Body Irradiation - UHBI

A 67 day old rabbit was starved overnight, anesthetized with halothane, ECG electrodes attached, and a catheter filled with heparinized 0.9% saline implanted in the dorsal aorta; all according to the methods explained in Materials and Methods D. After a recovery period lasting several hours, the animal was taken to the RRC and the aortic blood pressure, ECG, and ear temperature were recorded. The rabbit was then transported to the CCI and its internal anatomy was observed fluoroscopically. The area to be exposed was marked on both longitudinal sides of the lucite box. The Theratron 80 Co^{60} therapy unit was used to deliver 1200 cGy with a dose rate of 110-120 cGy/minute, at the portion of the body lying ahead of the most anterior portion of the diaphragm (recall that this structure is curved).

The rabbit was returned to the RRC and physiological recording was resumed for 121 minutes. The lowest post-irradiation pressure was 16.1% lower than the lowest pre-irradiation mean pressure. The ECG did not exhibit T-wave elevation, and the ear temperature did not show any marked, sudden rise. The rabbit was killed with pentobarbital at the conclusion of the recording session.

Appendix IV

Range of Variation in Percent Change of the Vagal Power Spectrum After TBI

Figure 54. The percent change in each frequency band of the vagal power spectrum after TBI, shown individually for each of the six rabbits which displayed ACC. The figure was prepared by first subtracting the microvolt value in each band of a pre-irradiation power spectrum photograph, from the microvolt values in the corresponding bands belonging to the power spectrum photographs which were taken at an average of 70 minutes after TBI. In other words, the data in this figure were averaged to create the 70 minute curve in Figure 28. The values produced by subtraction were divided by the pre-irradiation values, and each quotient was multiplied by 100 to yield percent change.

Figure 54: Percent Change in Power Spectrum for Six Acutes

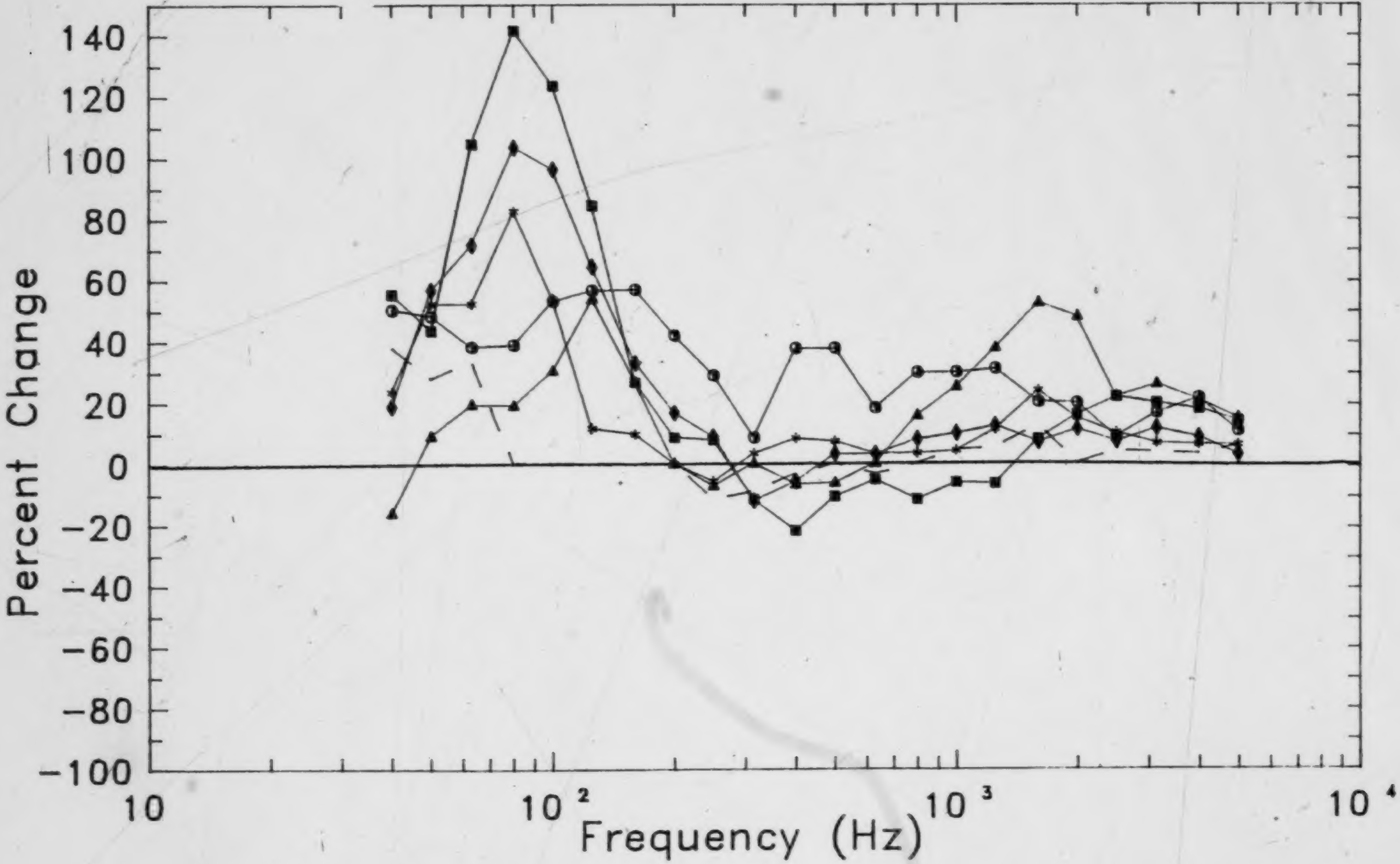

Figure 55. The per cent change in each frequency band of the vagal power spectrum after TBI, shown individually for two rabbits which exhibited deliberate hypotension. The data for the figure were prepared as in figure 54. The per cent change is shown for the average time of 80.5 minutes after TBI; ie, the data shown here was averaged to produce the 80.5 minute curve in Figure 29.

Figure 55: Percent Change in Nerve PS for Four Deliberates

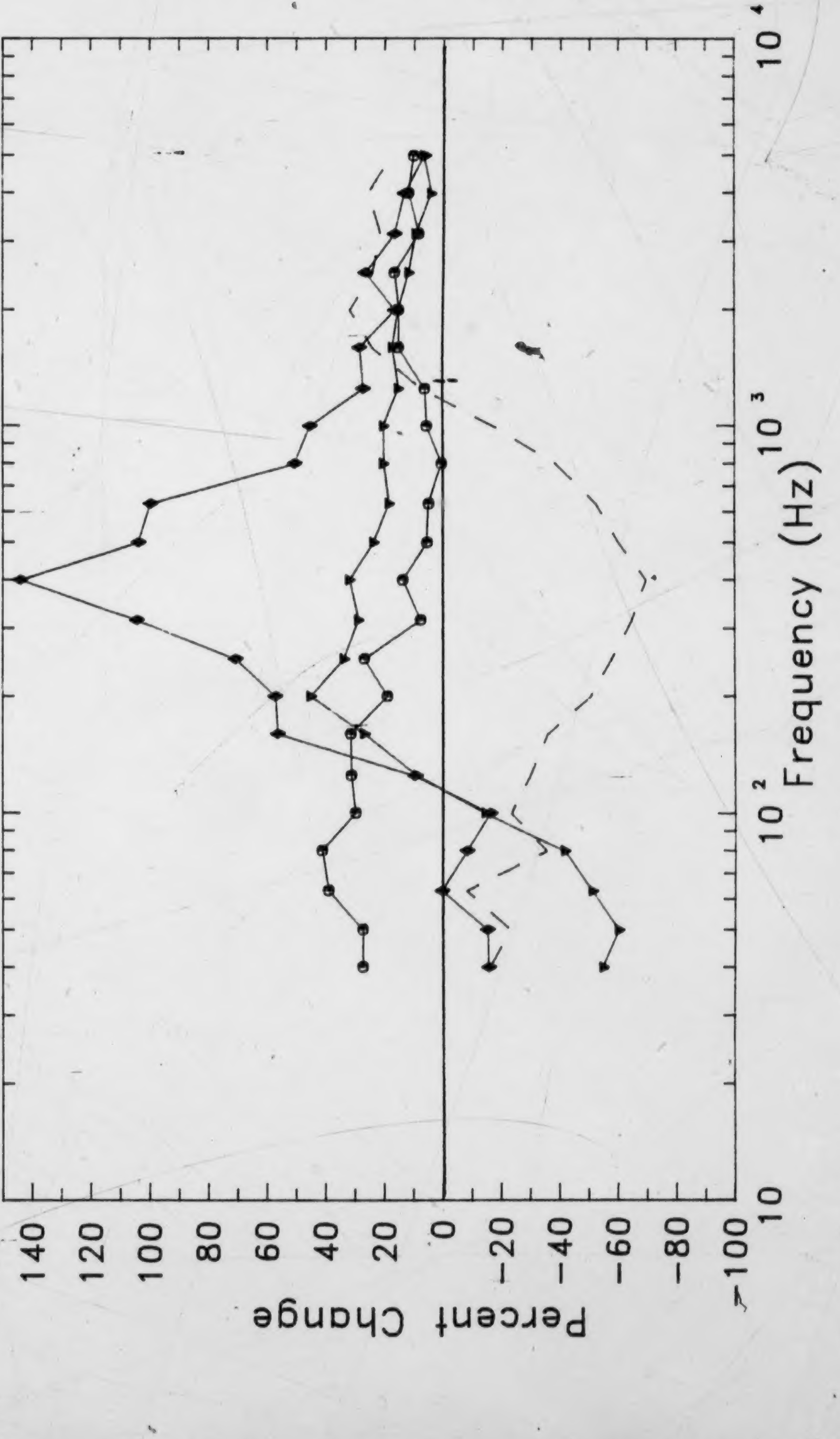

Figure 56. The per cent change in each frequency band of the vagal power spectrum after TBI, shown individually for four rabbits which displayed the deliberate response. These animals differed from those in Figure 55 in that their power spectra contained substantial voltage amplitudes in the middle frequency region. The per change is shown for the average time of 73 minutes post TBI. In other words the data presented here were averaged to construct the 73 minute curve in Figure 30.

Figure 56: Percent Change in Nerve PS for Two Deliberates